Self-assembly of cyclodextrins and their complexes in aqueous solutions

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ABSTRACT: Cyclodextrins are enabling pharmaceutical excipients that can be found in numerous pharmaceutical products world-wide. Due to their favorable toxicological profiles cyclodextrins are often used in toxicological and Phase I assessments of new drug candidates. However, at relatively high concentrations cyclodextrins can spontaneously self-assemble to form visible microparticles in aqueous mediums and formation of such visible particles may cause product rejections. Formation of sub-visible cyclodextrin aggregates are also known to affect analytical results during product development. How and why these cyclodextrin aggregates form is largely unknown and factors contributing to their formation are still not elucidated. The physiochemical properties of cyclodextrins are very different from simple amphiphiles and lipophilic molecules that are known to self-assemble and form aggregates in aqueous solutions but very similar to those of linear oligosaccharides. In general, negligible amounts of aggregates are formed in pure cyclodextrin solutions but the aggregate formation is greatly enhanced upon inclusion complex formation and the extent of aggregation increases with increasing cyclodextrin concentration. The diameter of the aggregates formed is frequently less than about 300 nm but visible aggregates can also be formed under certain conditions.

Keywords: cyclodextrin; aggregates; complexation; inclusion compounds;

Dr. Marcus Brewster

With this review paper, we would like to honor our esteemed and deeply respected colleague Dr. Marcus Brewster. He was Vice President and Scientific Fellow of the Pharmaceutical Development and Manufacturing Sciences group at Janssen Pharmaceutica R&D.

Dr. Marcus Brewster contributed to the development of several important medicines that are benefitting patients today. He was a major advocate for the use of cyclodextrins (CDs) in drug formulations and his contributions created the foundation for the regulatory approval and marketing of 2-hydroxypropyl-β-cyclodextrin (HPβCD)-based formulations worldwide including both oral and intravenous based dosage forms of itraconazole (Sporanox®). Thanks to his work, CDs are now routinely used in toxicological and Phase I assessments of new drug candidates.

The discovery of the self-association behavior of both CDs and certain drug-CD complexes sparked the interest of Dr. Marcus Brewster and it quickly became one of his favorite research topics. For a number of years we enjoyed collaborating with him on this subject. We had submitted a grant application to study self-assemble of CDs and CD complexes, and its impact on CD research and applications. It took couple of years but few months before his death we received a very generous grant to study “drug-CD aggregates in formulation assessment and drug delivery”. The subject of the grant originated from an observation of visible microparticles in parenteral solutions containing relative high CD concentrations. The formation of such visible and sub-visible CD aggregates may cause rejections and even product withdrawal. On the other hand, these interesting constructs can also be looked at as a starting point for a new breed of drug delivery vectors. These structures seem to form under certain conditions but are less common in other environments.

Drug-CD constructs hold the promise of formulating difficult-to-formulate drugs as well as specifically targeting their delivery topically, orally as well as parenterally. Initial studies have demonstrated that topical assessment of these systems to the skin resulted in targeted drug delivery to the hair follicles and sweat glands. In addition, work in modified Caco-2 cell cultures (e.g., mucus-producing systems) and other models have suggested that CD aggregates may act as mucus-penetrating delivery vectors that can

rapidly translocate drugs from the intestinal lumen to the absorptive epithelium. This effect has been reported to be enhanced by nanoparticles with hydrophilic coronas that minimize protein interaction.

How and why these aggregate systems form is largely unknown and factors contributing to their robustness are still not elucidated. The aggregation phenomena in CD solutions have been for a long time ignored by academic and industrial scientists. Only during the last decade several research groups in the world started to focus on this problem. This article describes in more detail the observations that lead to this grant application.

Introduction

Some water-soluble solute molecules spontaneously self-assemble in aqueous solutions to form aggregates.[1] Depending on the molecular structure and solvent properties, the aggregates may take a shape of rods, discs, prolate spheroids, spheres, bilayers, vesicles or reversible micelles.[2,3] At concentrations exceeding the critical aggregation concentration (CAC) a high degree of association may even lead to formation of lyotropic mesomorphs, e.g. lamellar, hexagonal, cubic and ribbon phases.[4-6] The self-assembly properties of substances can be used to develop novel pharmaceutical formulations. Different types of aggregates may be developed into drug delivery systems like micelles, nanoparticles, microspheres, liposomes and hydrogels to overcome formulation challenges, such as poor aqueous drug solubility, drug permeation through mucus and membrane barriers, and inadequate drug stability, as well as to enhance biological properties of drugs such as to reduce or prevent their side effects.[7-10] Application of molecular self-assemble in pharmaceutical technology and other sciences will however require thorough understanding of the thermodynamics and kinetics involved.

Surfactants and other amphiphiles, that have both hydrophilic and hydrophobic sections in their structure, are known to self-assemble in aqueous solutions to form micelles with a hydrophobic core and hydrophilic outer shell. Full understanding of the micelle formation and the forces involved (e.g., van der Waals, electrostatic and hydrophobic interactions, hydrogen and coordination bonds) is, however, still lacking.[1] Micelle formation is a thermodynamically favored process. The attraction of hydrophobic

species, resulting from their avoidance of water molecules in aqueous solutions, represents the hydrophobic interaction (i.e. formation of hydrophobic bonds). This interaction is favored thermodynamically because it minimizes the contact surface between water molecules and the non-polar group of an amphiphile with a consequent entropy increase (Fig. 1). The entropy increase (ΔS) upon micellization is partly due to increased flexibility of the hydrocarbon chains (on their transfer from an aqueous environment to the hydrophobic micellar core) and partly due to release of water molecules during neutralization of the ionic charge by the counterions (in case of ionic and zwitterionic surfactants).[11] Furthermore, there is an additional enthalpic contribution (ΔH) associated with the van der Waals interactions between hydrocarbon chains as well as between the hydrophilic head groups and the surrounding water molecules.

For many non-ionic micelles, the numerical values of the enthalpic contribution to the Gibbs energy of micelle formation ($\Delta G = \Delta H - T\Delta S$) is of the order of 10 kJ per mole, while the entropic term ($T\Delta S$) can have a value of about 40 kJ per mole.[12] Consequently some researchers have emphasized the significance of the entropic effect and hydrophobic interactions in the process of micelle formation.[13] Significance of the entropic components has been traced in atomistic simulations studies of the self-assemble of non-ionic chromonic molecules.[14] The calculations have showed that the numerical value of the entropic term is 1.5 times higher than that of the enthalpic one. Other researchers have studied thermodynamic properties of micellization of zwitterionic surfactants through molecular dynamics simulations and found that the aggregation process is entropy-driven and enthalpy–entropy compensated with increasing contribution of the enthalpy-driven part.[15,16] Based on an isothermal titration calorimetry study of homologous series of sulfobetaines (a form of zwitterionic surfactants), it was shown that the thermodynamics of micellization is mainly entropically driven at low temperatures but by both enthalpy and entropy at elevated temperatures. Such exchanging in dominant terms (from entropic to enthalpic) has been explained as reduction of water cohesion (or hydration ability) at elevated temperatures accompanied by consequent reduction of the hydrophobic effect.[13,17] Indeed, the free energy of aggregation involves significant changes in repulsive and attractive contributions of the

various interactions between hydrocarbon chains, head groups and solvent molecules. These interactions are equilibrium processes that occur on a microsecond timescale.

Self-assemble of solute molecules in aqueous solutions is affected by both internal and external factors. Internal factors include only native properties of the self-associated molecules while the external ones depend on environmental condition of the aqueous media such as temperature and ionic strength. Influence of the molecular structure and their physicochemical properties can easily be estimated from the numerous theoretical works. Some quantitative structure–activity relationship (QSAR) efforts describing the critical micelle concentration (CMC) are highlighted in a publication by Messina et al.[18] Frequently, the main descriptors are or depend on the size and surface area of the hydrophobic and hydrophilic moieties as well as on numerous other molecular properties such as the structural complexity of the hydrophobic moiety, van der Waals volume of the molecule, molecular flexibility, dipole moment of the molecule, relative positive charge and partial positive surface area of the hydrophobic domain, heat of formation and the octanol-water partition coefficient. For example, increased chain length of a hydrophobic moiety usually decreases the CMC value.[19-21] On the contrary, increasing the number of ionic head groups, or the charge of an ionic head group, will cause increase of electrostatic repulsion between them. That is why substances with ionic head groups (i.e. hydrophilic moieties) have higher CMC values than those containing non-ionic ones. Repulsive forces also define the size of micelles.[22] If the repulsive forces are strong the micelle size becomes small but it can become very large when the repulsive forces are weak as in the case of nonionic surfactants. Regarding external factors, increase of medium ionic strength will reduce solute hydration, decrease electrostatic repulsion and increase counterion binding that ultimately will decrease the CMC.[18,23] Changing of ionic strength is more effective for ionic substances than non-ionic ones. Influence of temperature on aggregation process is however not so ambiguous. Temperature dependencies of the CMC are frequently not linear but have U-shape.[19,20] The U-shape relationship is the product of two opposing effects observed during the temperature increasing, that is the decreasing in the hydration of the head groups and disordering of structured water surrounding the hydrophobic moieties. For

ionic surfactants the cohesion between the molecules becomes more difficult with increasing temperature and hence CMC increases.

The physicochemical properties of water

Understanding of solute self-assemble in aqueous solutions requires some understanding of the physicochemical properties of water and the solute-water interactions. Although the structure of liquid water appears to be trivial, consisting of two hydrogen atoms forming covalent bonds with one oxygen atom, its physicochemical properties are very unique.[24-27] The electrostatic surface of water is characterized by the difference in electronegativity of the hydrogen and oxygen atoms, 2.20 and 3.44, respectively, by the Pauling scale. Thus, the water molecule is a dipole with a partly negative oxygen atom and two partly positive hydrogen atoms. The result is electrostatic attraction between hydrogen atoms of one water molecule and the oxygen atoms of nearby water molecules, that is, to formation of H-bonds (i.e., hydrogen bonds) between neighboring water molecules. In liquid water the bond energy of the covalent H-O bonds is 492 kJ per mole while the bond energy of the intermolecular H-bonds is much lower or about 23 kJ per mole.[24] Traditional concepts of the structure of liquid water imply the presence of a complex hydrogen bond networks, where one molecule of water can have up to 4 hydrogen bonds (Fig. 2). However, recent studies show that at room temperature about 80% of water molecules make one strong H-bond and, by symmetry, accept one H-bond for a total of two H-bonds per water molecule. Furthermore, due to their relatively low activation energy the H-bonds in liquid water are constantly switching partners forming networks of water chains and rings that are continuously being formed and dissembled on the femtosecond (i.e., 10^{-15} of a second) timescale in weakly hydrogen-bonded disordered network.[28,29] Consequently, water behaves like a polymer ($(H_2O)_n$) of a number (n) of monomeric water units (H_2O) with a boiling point that is about 200°C higher than expected based on the boiling points of other group VI hydrides of the periodic table.[27] Although water and clear aqueous solutions appear homogeneous they are heterogeneous where solvent and solute clusters are constantly being formed and dissembled.[30] Solute molecules do affect hydrogen bonding of water.

Hydration and self-assemble of carbohydrates

Carbohydrates represent the most abundant class of organic compounds and play a fundamental role in many biological processes. Due to their physicochemical, structural and favorable toxicologic properties carbohydrates are commonly used as excipients in pharmaceutical, food and cosmetic products. Thus, aqueous carbohydrate solutions are of interest to pharmaceutical scientists as well as the interaction of carbohydrates with other solute and solvent molecules. Carbohydrates are organic compounds that have the highest number of hydroxyl groups per molecule and these hydroxyl groups can both serve as H-bond acceptors and H-bond donors. Molecular dynamics simulations have shown that the H-bonding abilities of glucose in aqueous solutions is both concentration and temperature dependent.[31] It is thought that at low concentrations glucose molecules are more efficient H-bond acceptors while at high concentration they are more efficient H-bond donors.

The ability to form H-bonds significantly affects the interaction between carbohydrate molecules such as sugars and between carbohydrate molecules and their hydration shell.[32-35] Hydration of sugars has recently been studied by both simulation (i.e. in silico)[32,36-38] and experimental[39-42] techniques. When monosaccharides and oligosaccharides are dissolved in water each OH group forms a H-bond with approximately three water molecules although the number of bound water molecules decreases somewhat with increasing molecular weight (Mw). Hence, in dilute aqueous solutions one glucose molecules binds 14 water molecules while maltodextrin molecule, containing on the average 6.2 glucose units, binds 50 water molecules or about 8 for each glucose unit.[39] It has been estimated that each glucose unit of α-cyclodextrin (αCD) and γ-cyclodextrin (γCD), formed by 6 and 8 glucose units, respectively, binds about 6 water molecules in aqueous solutions at 25°C.[43] However, the hydration value (i.e., the number of bound water molecules) does depend on the method applied and this observed variation in the hydration value is frequently explained by specific interactions of solute molecules due to sharing of hydration shells.[36] In other words, the tendency of sugars and other carbohydrates to aggregation even at low concentrations leads to increasing of the so-called hydration-water-sharing phenomenon (Fig. 3). Binding of water molecules decreases their mobility that leads to increased media viscosity with increasing sugars concentration. At low sugar concentrations the mobility

of water molecules decreases (i.e., the solution viscosity increases) linearly with increasing sugar concentration but then the viscosity increases more rapidly with positive deviation from linearity as the sugar concentration increases due to overlapping of the hydration shells and cluster formation.[36,44] CDs appear to display similar behavior (Fig. 4). Studies of aqueous sugar solutions have shown how sugar molecules influence the arrangement of water molecules.[45-47] At lower concentrations they behave as a water structure breakers, while at higher concentrations they act as a structure makers. In fact, studies indicate that increasing sugar concentrations lead to overall increase in number of H-bonds between sugar molecules and water, as well as to increased water-water H-bond lifetimes and the associated activation energies with a consequent viscosity increase.[48-50]

Frequently the tendency of sugar molecules to aggregate is associated with their ability to form intermolecular H-bonds.[40,42,51] Glucose, trehalose, maltodextrin and other carbohydrates are known to self-associate in aqueous solutions and undergo concentration-dependent clustering.[32,44,52-54] For example, a recent study of sugar solutions by dynamic light scattering (DLS) revealed power law growth of glucose, maltose and sucrose clusters as a function of sugar concentration.[53] Due to strong intermolecular H-bonding such clusters can be stable at temperatures as high as 90°C.[53,55] Simulations have shown that trehalose molecules form clusters in aqueous solutions and that their sizes increase with increasing trehalose concentration (Fig. 5) with a percolation concentration (i.e. the concentration at which continuous three-dimensional trehalose lattice is formed) of about 70% (w/v).[51] Small clusters are formed at low trehalose concentrations (i.e. less than 1% w/v) and these small clusters are short-lived on the picosecond scale, constantly being formed and dissembled.[40] Aminocellulose (Mw 3250 Da) has been shown to form fully reversible tetramers in aqueous solutions through H-bonding and that these tetramers associate further in a step-wise fashion into higher order structures.[56] Indeed, with increasing sugar concentrations the average number of H-bonds between individual sugar molecules increases until continuous H-bonded carbohydrate networks are formed in the aqueous media.[51,53,54] The observed differences in aggregation behavior of the various sugars, such as sucrose, maltose and trehalose, can be explained by their structural differences

and their abilities to form intra- and intermolecular H-bonds.[37,42,48] Topological (i.e. three-dimensional structural) differences of these molecules and their ability to form intra- and intermolecular H-bonds determines their ability to aggregate and the size of the aggregate clusters formed as well as their influence on the dynamics of water molecules of the hydration shell.

Self-assemble (aggregation) of cyclodextrins

The four main chemical groups of carbohydrates (synonym: saccharides) are monosaccharides, disaccharides, oligosaccharides (typically formed by 3 to 9 monosaccharides) and polysaccharides. CDs form a subgroup of oligosaccharides that were discovered over 120 years ago by Antoine Villiers (1854-1932) during his study of enzymatic degradation of carbohydrates.[57-59] In the following decades the cyclic structure and chemistry of CDs was revealed and shown that CDs consist of several α(1→4) linked D-glucopyranose units (Table 1).[60,61] The arrangement of hydroxyl groups on the doughnut-shaped CD molecules bestows hydrophilic and hydrophobic domains on them: a polar exterior and a non-polar interior. While their polar exterior allows for solvation of CDs in aqueous solutions their central cavity enable encapsulation of non-polar molecules or molecular moieties, a property that has been explored in supramolecular,[62-66] analytical,[67-71] food[72-75] and pharmaceutical[76-81] chemistry.

The structure and physicochemical properties of CDs (Table 1) are similar to those of other oligosaccharides but very different from the previously mentioned simple amphiphiles and hydrophobic drugs (Fig. 1). CDs are carbohydrates and do self-assemble like other carbohydrates. The best known example of such self-assemble in aqueous solution is probably opalescence of aqueous γCD solutions followed by γCD precipitation that occurs even at low γCD concentrations. This has hampered the usage of γCD in pharmaceutical products such as parenteral solutions. Szente and his coworkers tried to explain and prevent this γCD aggregation.[82] Their conclusion was that the only practical way to prevent opalescence of aqueous γCD solutions was through chemical modifications of the γCD molecule. During studies of aqueous CD solutions, even aqueous solutions of some of the very water-soluble CD derivatives, investigators frequently noted that their behavior did not follow accepted theoretical equations and

sometimes indicated that observed deviations from theoretical equations could be due to formation of CD aggregates. For example, Miyajima and coworkers[83,84] observed that the activity coefficient of aqueous CD solutions decreases with increasing concentration of αCD, γCD and dimethyl-βCD, and explained this observation by CD dimerization. Self-assemble of CD molecules has also been suggested based on X-ray[85-90] and scanning tunneling microscopical data,[91] as well as by molecular simulation studies.[92-94] A complex concentration and temperature dependency of the activity coefficient of aqueous CD solutions was observed by Terdale coworkers and related to both CD–water and CD–CD interactions.[95] Studies indicated that the presence of H-bonded CD aggregates in aqueous media and their interactions with water molecules were responsible for the unexpected CD behavior.[96-99] Numerous studies by our group show how micelle-like assembles of dissolved CD molecules and their complexes affect CD solubilization of lipophilic drugs in aqueous media and how these assembles hamper drug permeation through semi-permeable membranes.[100-104]

The growing industrial applications of CDs and improvements in analytical technology have sparked interest in self-assembled CD aggregates. Formation of such aggregates in aqueous solutions is now generally accepted although there are still some disagreements regarding to their size and shapes.[105,106] Numerous light scattering studies have shown that the parent αCD, βCD and γCD form aggregates in aqueous solutions.[96,107-110] Some of these results have been summarized and presented as plots showing the relationship between the aggregate size and CD concentration (Fig. 6).[105]

For known self-assembling compounds like ionic liquids or surfactants, the CAC (or CMC for surfactants) is usually determined by measurement of physicochemical parameters of their aqueous solutions as function of compound concentration. Frequently used methods are tensiometry, conductometry, fluorimetry, light scattering, or nuclear magnetic resonance (NMR) spectroscopy.[111-117] The common procedure to determine the CAC from experimental data is to determine the intersection of two straight lines traced through plots of the measured physical property versus the concentration. For CDs and CD complexes, there are few publications that describe the determination of the CAC. In a study of βCD self-assembly by De Sousa et al., scattering intensity was monitored by DLS during titration of βCD into water.[118] Two

distinct regions were observed when the scattering intensities as a function of βCD concentration were plotted. The CAC values of βCD at different temperatures were determined and used to calculate the thermodynamic parameters (Gibbs energy, enthalpy, entropy) of the formation of βCD aggregation.

Determination of CAC with different analytical techniques can lead to different values. In one study, the chemical shift of the CH_3 group of HPβCD was measured from ^{1}H-NMR spectra obtained at different concentration ranges.[119] In this study, the chemical shifts were plotted as a function of the inverse of the total HPβCD concentration. The calculated CAC for HPβCD was 77.7 mg/ml. In another study, conductivity was used to obtain the CAC value of HPβCD by plotting the conductivity values against the HPβCD concentrations in aqueous solution.[120] In that case the CAC of HPβCD was determined to be 69.3 mg/ml. Similar differences have also been observed during aggregation studies of ionic liquids in aqueous media or of CMC of surfactants. The reason for these differences in determined CAC values is, first, different techniques measure different physicochemical parameters and, second, the CAC is rather a concentration range at which the aggregation of free monomers begins rather than one specific concentration below which no aggregates can be found and above which one specific type of aggregates exists.[120,121]

The general trend is that the aggregate size increases with increasing CD concentration. Moreover, the aggregates appear at CD concentrations as low as 3-12 mM, that is well below the CD solubility of 150 (αCD), 16 (βCD) and 180 (γCD) mM.[122] Furthermore, the studies show size distribution that can vary from 20 nm to a couple of μm although the reported diameter of CD aggregates is most frequently between 90 and 300 nm. The kinetics of aggregate formation is not well understood and aggregate size variations have been observed, even during repeated size determinations of identical CD solutions by identical methodology.[82,107] CD molecules may lose hydrogen-bond donors (i.e., OH groups) upon derivatization and generally consist of not one molecular structure one but of a complex mixture of geometrical and optical isomers and, thus, CD derivatives are thought to have less tendency to self-assemble and form aggregates than their parent CDs.[105,107,108,123]

The DLS technique, which is frequently used to detect nanoparticles, assumes that the self-assembled aggregates have spherical structure while the various microscopic methods allow observation of the actual shape of aggregates. Studies of aqueous βCD solutions by transmission electron microscopy (TEM) at cryogenic temperature confirmed the concentration dependency of the mean aggregate diameter but revealed also that the shape of CD aggregates is concentration dependent.[109] At βCD concentration of 3 mM small (6 nm) globular particles were observed that could interact with each other to form branched and discoidal structures with size ranging from 30 to 100 nm. Increasing the βCD concentration above 6 mM leads to the formation of new structures, large (over 500 nm) sheet like domains, which can be transformed to fibers and folded lamellae by sonication. Stirring of the aqueous media has been shown to affect the shape of the aggregates.[124] A Cryo-TEM study of amphiphilic βCD derivatives showed spheroidal particles with a diameter of approximately 50 nm.[125] Kinetic measurements indicated that the nano-spheres formed are stable for at least three years. Porous silica material has been prepared using various parent CDs and their derivatives as template phases.[126] Microscopical studies of the products displayed formation of worm-like cavities occupied by supramolecular CD aggregate structures. More detailed review of microscopy characterization of CD assembles was published by He and coworkers.[106]

It is noteworthy that the wide variety of shapes formed by CD aggregates is based on only two types of molecular arrangements. If we consider the association processes as the nucleation step during crystallization then molecular packing during nucleation and crystallization should be identical. Crystal structures of both native CDs and their inclusion complexes are either of channel- or cage-type (Fig. 7).[89] Rapid recrystallization of αCD and γCD results in formation of channel structures.[127,128] It was shown that induced water sorption-desorption cycle results in phase transformation (from channel to cage type) with intermediated amorphous state. After reaching sorption equilibrium, the CD molecules undergo a slow rearrangement (duration of about 100 h for αCD and 192 h for γCD) to the cage structure with defined water content. These observations show how important water molecules are and how hydration shell affects the structure of self-assembled CD molecules, and the structure and dimensions of CD aggregates.

The observations and experimental results described above show how complex and ambiguous self-assemble of CD molecules and their aggregate formation is. Indeed, self-assemble of CD molecules in aqueous solutions to form aggregates and disassemble of the CD aggregates formed are in dynamic equilibrium. Moreover, the encapsulated water molecules present in the CD cavity and water molecules forming their hydrated shell are in dynamic equilibrium with other water molecules in the aqueous media on the nanosecond timescale. In turn, every CD molecule has its own dynamic behavior caused by rotational freedom of the glucopyranose building blocks and the overall molecular mobility. Stabilization of these aggregated systems is controlled by specific (van der Waals forces) and non-specific (hydrogen bonds) interactions, which are at all times in competition. Although the ability of CDs to self-assemble to form aggregates is well documented it has also been shown that the aggregates are very unstable. Attempts to stabilize nano-size self-assembled CD aggregates of the native αCD, βCD and γCD and their hydrophilic (monomeric) derivatives have so far not been successful.

Self-assemble of cyclodextrin complexes

CD inclusion complex is formed when lipophilic molecule (the guest), or more frequently some lipophilic moiety of a larger molecule, enters the somewhat hydrophobic central cavity of a CD molecule (the host). A number of forces are thought to be involved in the inclusion complex formation including van der Waal's forces, electrostatic interactions, formation of H-bonds, release of conformational strain and exclusion of high energy water bound in the CD.[129-131] The complex formation is almost always associated with a relatively large negative ΔH and a ΔS value that can either be positive or negative. In addition to inclusion complexes CDs are, like non-cyclic oligosaccharides, also able to form non-inclusion complexes where, for instance, the hydroxyl groups on the outer surface of the CD molecule form hydrogen bonds with the drug of interest.[130] For example, using mass spectrometry (MS) it has been shown that αCD forms both inclusion and non-inclusion complexes with dicarboxylic acids and that the two types of complexes coexist in aqueous solutions. MS allowed selective measurement of CDs as well as drug/CD complexes, including their stoichiometry. Distinction between inclusion

and non-inclusion complexes was facilitated by comparison with interaction of the drug with a linear sugar incapable at making inclusion complexes in solution. This way the obtained MS data provided a valuable image of the two types of complexes that coexist in aqueous solution.[132]

Over the years a wealth of data has been accumulated about CD complexes and their physicochemical properties. The complexation changes the physicochemical properties of both the guest and the host, and both physicochemical and biological properties of drugs can be improved through CD complexation. Numerous research publications and reviews have reported how drug solubility,[133-138] permeability,[26,133,139,140] bioavailability[74,141-144] and chemical stability[145-147] can be improved through CD complexation. Most often drug/CD complexes are dimer complexes (i.e., formed by one drug molecule and one CD molecule) but ternary complexes are also frequently described where water-soluble polymers, metal ions or organic salts are used to enhance the CD effect.[148] It should be emphasized that in aqueous solutions there is a constant equilibrium between molecules forming the CD complex and free dissolved molecules. Furthermore, more than one type of guest/CD complexes can coexist in aqueous solutions. For instance, in aqueous solutions vanillin has been shown to form 1:1 inclusion complex with βCD but 1:2 vanillin:βCD complexes were also detected.[149] In multicomponent systems, containing more than one type of guest molecules, reorganization of complexes can be initiated by competition among different types of guest molecules and in a dynamic system complex composition can change over time.[150,151] Figure 8 shows a schematic representation of different transformation pathway of a drug-CD system. The figure shows that interactions between drug molecules and CD molecules are not limited to inclusion complex formation. Coexistence of inclusion and non-inclusion complexes in aqueous CD solutions has been documented[101,102,132,152-155] and there are some indications that formation of such non-inclusion complexes can be associated with formation of drug/CD complex aggregates.[156] In aqueous solutions free CD molecules have some tendency to self-assemble to form aggregates. However, their tendency to self-assemble increases upon formation of inclusion complexes and appears to be proportional to the concentration of inclusion complexes in the solution (Fig. 9).[157] For example, the asymmetry of Job's

plots obtained by NMR studies of aqueous CD solutions has suggested premicellar association of inclusion complexes of a cationic surfactant and βCD followed by micellar association of the inclusion complexes.[158] Other investigators observed micelle-like assembles (micelle structures with diameters more than 200 nm) in aqueous solutions containing trans-β-carotene and βCD and γCD.[159] Previously we studied the effects of CD concentrations on the permeation of hydrocortisone,[100,160-162] indomethacin,[163] diclofenac sodium,[161,163] amphotericin B,[161] irbesartan,[164] carvedilol,[165] dexamethasone[163,166] and dorzolamide[167] through semi-permeable cellophane membranes of various molecular weight cut-offs (MWCO). The observed permeation profiles displayed negative deviation from the expected linear profiles predicted by Fick's first law of diffusion. Formation of drug/CD complex aggregates in the nano-size range was confirmed by other techniques such as DLS and TEM, and the observed negative deviation from linearity shown to be due to drug/CD complex aggregation. In most cases the drug/CD complex aggregation was initiated at CD concentration of about or below 10% (w/v). Self-association of CD complexes can explain the observed decrease in the activity coefficient with increasing of CD concentration,[161] and the method dependent complex stoichiometry (i.e., the stoichiometry of a given complex depends on the method applied).[101,104] These observations are summarized in Table 2.

Amphiphilic CDs have been obtained by chemical modifications of their macrocycles using various anchors (e.g. phospholipidyl, cholesteryl and oligo(ethylene oxide) groups). Grafting of hydrophobic moieties by reaction with the primary or secondary hydroxyl groups confers them an amphiphilic character.[168-172] Such amphiphilic compounds may self-organize in aqueous phase to form supramolecular assemblies. One such example is Chol-DIMEB: this molecule consists of a parent methylated βCD in which one of the anhydroglucose units is linked with cholesterol via its primary hydroxyl group through a succinic acid spacer. The molecule self-aggregates into micellar structures of about 5 nm in diameter with an aggregation number of about 24 and a CMC of $5\pm2\cdot10^{-6}$ M, indicating a high associative behavior.[169] The micelles form a hydrophobic core (the cholesterol moiety) and a polar surface (methylated βCD).

Effects of derivatization, ions, polymers and other excipients on the complexation and aggregation

Water-soluble polymers and surfactants are known to stabilize various types dispersed systems in aqueous solutions.[173-176] Similarly, water-soluble polymers, salts and surfactants have been shown to have stabilizing effect on CD aggregate and, in some cases, to enhance the complexation efficiency and solubility of drug/CD complexes.[156,177-185]

Substituted CDs have been specifically designed to improve aqueous solubility. These CD derivatives often present mixtures of positional and regional isomers with different degree of substitution (DS). Interestingly, it has been demonstrated that the DS of CD derivatives influences the entropy and enthalpy driven processes which contribute to drug inclusion formation, eventually causing changes in the complexation capability of CDs. For example, it has been reported that the affinity of different substrates for SBEβCD is affected by the DS. Notably, depending on the nature of the guest molecule, the affinity may be higher or lower with increasing DS.[186] Moreover, the presence of substituents interferes with the carrier performance of drug/CD complexes, as demonstrated by Mennini and co-workers.[187] The nature of the inclusion is highly specific and a proper characterization could shed light on the complex mechanisms involved in both CDs complexation and aggregation phenomena.

The behavior of cholesterol/CD complexes in the presence of glucose, urea and inorganic salts has been investigated and does further illustrate the effect of additives on aggregation. Cholesterol, solubilized in 1 and 3.3% CD solutions displayed a mixture of non-aggregated complexes with diameter of 1-2 nm and aggregated complexes with diameter of 100-1000 nm. The effect of additives such as glucose, urea and ionic salts on the fraction of aggregated complex did depend on the type of CD. While the additives enhanced the fraction of aggregated complexes for random methylated βCD in a synergistic way, the opposite effect was noticed in the case of HPβCD where the fraction of aggregates also depended on the molar substitution degree.[188]

Effects of cyclodextrin aggregation on analytical results

Numerous analytical techniques are applied to investigate CD and CD complexes,[189-194] but in almost all cases aggregation of CD and CD complexes are not accounted for and if existence of such aggregates is acknowledged they are generally referred to as some minor event. However, in recent years it has been observed that CD aggregation and aggregation of CD complexes can affect analytical results and that the different analytical methods do not give consistent results or results that can be explained by the conventional thinking of guest/host complex formation.[101,102,123] For example, phase-solubility studies do frequently suggest formation of higher order drug/CD complexes with 1:2 or 2:1 stoichiometry although other analytical methodologies such as Job's plots and docking studies clearly show that only 1:1 drug/CD complexes can be formed. Likewise, the numerical values of drug/CD formation constants (e.g. $K_{1:1}$ stability constants of 1:1 drug/CD complexes) should be independent of both the drug and CD concentration as well as of the method applied to determine their values but frequently they are not. Lipophilic compounds that are known to have high affinity for the CD cavity should reduce CD solubilization of poorly soluble drugs but sometimes they actually enhance the solubilization. Again, these discrepancies and unexpected observations can be explained by formation of nano-size drug/CD complex aggregates that are able to solubilize poorly soluble drugs through micellar type solubilization.

The gas phase chemistry involved in MS detection offers the possibility to investigate complex formation without the complicating effect of solvent molecules. On the other hand, detection of inclusion complexes and/or CD aggregates in gas phase does not necessarily reflect the exact situation in solution phase.[195,196] A combined approach of different analytical techniques will often be required in order to obtain unambiguous information on conformation, kinetics and thermodynamics of drug/CDs complexes and aggregates in different phases.[197,198]

Application of CD aggregates as drug delivery systems

As previously discussed drug/CD complexes self-assemble in aqueous solutions to form water-soluble nanoparticles (i.e. aggregates) with a diameter that is frequently a couple of hundred nm, and these nanoparticles self-assemble further to form larger aggregates (i.e., forming colloidal, opalescent solutions) and microparticulate systems. Aqueous CD

solutions containing water-soluble CD complexes and aggregates are equilibrium systems where complexes are continuously being formed and dissociated in aqueous solutions at rates close to the diffusion-controlled limit (Fig. 9).[199] The nanoparticulate aggregates are unstable under normal conditions and dissociate almost instantly upon media dilution while the microparticular aggregates are somewhat more stable although they can dissolve quite rapidly. In fact, CD complexes and their aggregates tend to behave more like nanoscale systems, such as nano-suspensions, microemulsions and liposomes, rather than true solutions.[123,156] Self-assembled CD nanoparticles can have the drug delivery properties of nanoparticles but from a regulatory standpoint these delivery systems consists of water-soluble CDs that possess the same favorable pharmacokinetic and toxicological profile as the CD monomers.[200] This unique property of CD drug delivery systems has been explored in topical drug delivery to the eye, including targeted drug delivery to the eye retina, topical drug delivery to the hair follicles and microparticular enhanced drug delivery through mucus.[123,139,201,202]

Challenges and opportunities

As recent publications indicate, exploiting nanoaggregate formation has created new opportunities in drug delivery.[203,204] Although mostly topical formulations have been under scrutiny, the use of CD nanoparticles in oral drug delivery could be a promising strategy to improve the bioavailability of poorly soluble drugs. However, little is known about the behavior of these nanoaggregates under conditions that are representative for the gastrointestinal tract. Essential factors that may cause deviations from the observations made in pure water include the peculiar intraluminal hydrodynamics and the complex and continuously changing composition of the intraluminal environment to which the formulation is exposed. Implementation of these biorelevant conditions poses some challenges to the evaluation of the performance of the nanoaggregates.

Upon oral administration, a drug formulation is exposed to a highly dynamic environment. Gastric and intestinal contractions generate mixing and propulsion of the intestinal contents. Since the forces that are involved in the CD complex aggregation are relatively weak, disassembly of the complexes might occur, governed by the hydrodynamics existing within the stomach and the small intestine. Messner et al.

evidenced a strong decrease in the size of the aggregates upon stirring of an HPβCD containing aqueous medium, saturated with hydrocortisone.[205] In this set-up, continuous stirring was provided by a magnetic bar to demonstrate the relatively weak forces needed to induce disaggregation. As the relevance of continuous stirring for the *in vivo* situation is highly questionable, it would, however, be premature to conclude that CD nanoaggregates cannot exist in the intraluminal environment.[206,207] In the same study, Messner et al. also demonstrated a decrease in size of the CD aggregates in function of increasing temperatures. Nevertheless, the decrease in total fraction of aggregated complexes when comparing 10°C to 36°C was limited.[205] Consequently, it appears that aggregation can still occur at body temperature.

A third mechanism which may cause disaggregation is dilution of the formulation as it travels through the gastrointestinal tract.[208] Recent studies utilizing non-invasive imaging techniques or *in vivo* sampling from stomach and duodenum have generated insight into the *in vivo* behavior of a formulation, including information on the dilution factor upon gastrointestinal transit.[209-211] Further investigations could provide useful estimates of the concentrations of CDs present at different sites along the gastrointestinal tract.

Clearly, several physiological mechanisms may contribute to disassembly of CD nanoaggregates and diminished drug solubilization. It is important to note that this decrease in solubilizing capacity does not necessarily have to result in drug precipitation, as CDs have shown the ability to stabilize drug supersaturation.[156]

Since the introduction of simulated intestinal fluids, intestinal disposition studies have been slowly moving away from using aqueous buffers in favor of more biorelevant fluids.[212] As the pH of these media matches the values measured in stomach and small intestine, the ionization of the guest compound (and possibly of the CD molecule) becomes relevant. Ionization of the guest compound influences complex formation and this has been reported to affect self-assembly.[157] Consequently, characterization of CD aggregation at different biorelevant pH values is therefore imperative to understand *in vivo* behavior of the CD containing formulation upon oral administration.

In addition to the effect of pH on ionization, the presence of bile salts and phospholipids introduces additional complexity to the evaluation of CD self-assembly. It has been shown that both bile salts and phospholipids can act as guest molecules in CD

complexes[213-215] As mentioned earlier in this review, in multicomponent systems, competition between different guest molecules may result in reorganization of complexes and in dynamic systems, such as the intestinal environment, complex composition (and, consequently, CD aggregation) can change over time. As a result, the aggregation behavior and overall performance of the formulation in biorelevant media and in pure water may strongly differ. Therefore, the road towards the application of CD nanoparticles as a formulation strategy for oral drug delivery would have to include a thorough understanding of the behavior of CDs in biorelevant media.

A number of analytical tools are already in place to characterize drug-CD complexes and their aggregates. Somewhat surprisingly, so far MS has not been used for the characterization of CD aggregates. Over the last decade MS has developed into a key technique for the analysis of biomacromolecules, including proteins and their complexes. Ionization techniques allowing transfer of intact macromolecular assemblies into the gas phase have been established.[216] Based on the ongoing advances in resolution and sensitivity,[217] we expect MS to develop into a viable technique for the analysis of CD assemblies.

As compared to characterization in water, optimizing the methods for application in more complex media may prove to be challenging. Interaction between the CDs and constituents of the biorelevant media may impede the interpretation of analytical tests. Moreover, with respect to visualization, both colloidal structures consisting of bile salts and lipids and the CD aggregates have been reported to take different shapes, spanning overlapping size ranges. Obviously, this could hamper the interpretation of microscopic images, for example.

Further development and optimization of analytical methods can provide reliable information on the CD aggregation phenomenon under different biorelevant conditions.

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References

1. Whitesides GM, Grzybowski B 2002. Self-Assembly at all scales. Science 295(5564):2418-2421.
2. Daful AG, Avalos JB, Mackie AD 2012. Model shape transitions of micelles: spheres to cylinders and disks. Langmuir 28(8):3730-3743.
3. Verma G, Kumar S, Schweins R, Aswal VK, Hassan PA 2013. Transition from long micelles to flat bilayers driven by release of hydrotropes in mixed micelles. Soft Matter 9(17):4544-4552.
4. Zahid NI, Abou-Zied OK, Hashim R, Heidelberg T 2012. Fluorescence probing of the temperature-induced phase transition in a glycolipid self-assembly: hexagonal <-> micellar and cubic <-> lamellar. Langmuir 28(11):4989-4995.
5. Shearman GC, Brooks NJ, Tiddy GJT, Sztucki M, Templer RH, Law RV, Ces O, Seddon JM 2011. A lyotropic inverse ribbon phase in a branched-chain polyoxyethylene surfactant: pressure effects. Soft Matter 7(9):4386-4390.
6. Raoul Z. 2005. Introduction to surfactants and surfactant self-assemblies. Dynamics of surfactant self-assemblies: micelles, microemulsions, vesicles and lyotropic phases, ed.: CRC Press. p 1-35.
7. Hassani LN, Hendra F, Bouchemal K 2012. Auto-associative amphiphilic polysaccharides as drug delivery systems. Drug Discov Today 17(11-12):608-614.
8. Gong J, Chen M, Zheng Y, Wang S, Wang Y 2012. Polymeric micelles drug delivery system in oncology. J Control Release 159(3):312-323.
9. Roesler A, Vandermeulen GWM, Klok H-A 2012. Advanced drug delivery devices via self-assembly of amphiphilic block copolymers. Adv Drug Deliver Rev 64:270-279.
10. Parveen S, Misra R, Sahoo SK 2012. Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. Nanomedicine 8(2):147-166.
11. Tadros T. 2013. Micelle formation. In Tadros T, editor Encyclopedia of colloid and interface science, ed., Berlin: Springer Berlin Heidelberg. p 686-686.
12. Atkins P, de Paula J. 2010. Materials 1: macromolecules and self-assembly. In Atkins P, de Paula J, editors. Atkins' Physical chemistry, 9 ed., United Kingdom: Oxford University Press. p 676.
13. Chandler D 2005. Interfaces and the driving force of hydrophobic assembly. Nature 437(7059):640-647.
14. Akinshina A, Walker M, Wilson MR, Tiddy GJT, Masters AJ, Carbone P 2015. Thermodynamics of the self-assembly of non-ionic chromonic molecules using atomistic simulations. The case of TP6EO2M in aqueous solution. Soft Matter 11(4):680-691.
15. Liu G, Gu D, Liu H, Ding W, Luan H, Lou Y 2012. Thermodynamic properties of micellization of sulfobetaine-type zwitterionic gemini surfactants in aqueous solutions - a free energy perturbation study. J Colloid Interf Sci 375:148-153.
16. Brinatti C, Mello LB, Loh W 2014. Thermodynamic study of the micellization of zwitterionic surfactants and their interaction with polymers in water by isothermal titration calorimetry. Langmuir 30(21):6002-6010.
17. Angarten RG, Loh W 2014. Thermodynamics of micellization of homologous series of alkyl mono and di-glucosides in water and in heavy water. J Chem Thermodyn 73:218-223.
18. Messina PV, Besada-Porto JM, Ruso JM 2014. Self-assembly drugs: from micelles to nanomedicine. Curr Top Med Chem 14(5):555-571.
19. Inoue T, Yamakawa H 2011. Micelle formation of nonionic surfactants in a room temperature ionic liquid, 1-butyl-3-methylimidazolium tetrafluoroborate: surfactant chain length dependence of the critical micelle concentration. J Colloid Interf Sci 356(2):798-802.

20. Inoue T, Kawashima K, Miyagawa Y 2011. Aggregation behavior of nonionic surfactants in ionic liquid mixtures. J Colloid Interf Sci 363(1):295-300.
21. Lin L-H, Ye Y-W, Chen K-M 2013. Oxypropylene chain length affects the physicochemical properties of ortho ester-based gemini surfactants. Colloid Surface A 416:1-8.
22. Oliver RC, Lipfert J, Fox DA, Lo RH, Doniach S, Columbus L 2013. Dependence of micelle size and shape on detergent alkyl chain length and head group. Plos One 8(5).
23. Wang R, Li Y, Song Y, Zhi L 2014. Effects of ph, temperature and ionic strength on aggregation behavior of dodecyldiethoxylamine oxide. J Surfactants Deterg 17(2):223-229.
24. Chaplin M. 2011. The water molecule, liquid water, hydrogen bonds, and water networks. In Le Bihan D, Fukuyama H, editors. Water - the forgotten biological molecule, ed., Singapore: Pan Standford Publishing Pte. Ltd. p 3-19.
25. Griffith JH, Scheraga HA 2004. Statistical thermodynamics of aqueous solutions. I. Water structure, solutions with non-polar solutes, and hydrophobic interactions. J Mol Struc-Theochem 682(1-3):97-113.
26. Loftsson T 2012. Drug permeation through biomembranes: cyclodextrins and the unstirred water layer. Pharmazie 67(5):363-370.
27. Loftsson T, Brewster ME 2008. Physicochemical properties of water and its effect on drug delivery - a commentary. Int J Pharm 354(1-2):248-254.
28. Eaves JD, Loparo JJ, Fecko CJ, Roberts ST, Tokmakoff A, Geissler PL 2005. Hydrogen bonds in liquid water are broken only fleetingly. P Natl Acad Sci USA 102(37):13019-13022.
29. Head-Gordon T, Johnson ME 2006. Tetrahedral structure or chains for liquid water. P Natl Acad Sci USA 103(44):16614-16614.
30. Wakisaka A, Matsuura K 2006. Microheterogeneity of ethanol-water binary mixtures observed at the cluster level. J Mol Liq 129(1-2):25-32.
31. Chen C, Li WZ, Song YC, Weng LD, Zhang N 2012. Hydrogen bonding analysis of hydroxyl groups in glucose aqueous solutions by a molecular dynamics simulation study. B Kor Chem Soc 33(7):2238-2246.
32. Chen C, Li WZ, Song YC, Weng LD, Zhang N 2012. Formation of water and glucose clusters by hydrogen bonds in glucose aqueous solutions. Comp Theor Chem 984:85-92.
33. Te JA, Tan M-L, Ichiye T 2010. Solvation of glucose, trehalose, and sucrose by the soft-sticky dipole-quadrupole-octupole water model. Chem Phys Lett 491(4-6):218-223.
34. De Vito F, Veytsman B, Painter P, Kokini JL 2015. Simulation of the effect of hydrogen bonds on water activity of glucose and dextran using the Veytsman model. Carbohyd Polym 117:236-246.
35. Chen C, Li W-Z, Song Y-C, Weng L-D 2011. Structure and kinetics of hydrogen bonds in aqueous glucose solutions. Acta Phys-Chim Sin 27(6):1372-1378.
36. Fioretto D, Comez L, Corezzi S, Paolantoni M, Sassi P, Morresi A 2013. Solvent sharing models for non-interacting solute molecules: the case of glucose and trehalose water solutions. Food Biophys 8(3):177-182.
37. Magno A, Gallo P 2011. Understanding the mechanisms of bioprotection: a comparative study of aqueous solutions of trehalose and maltose upon supercooling. J Phys Chem Lett 2(9):977-982.
38. Pomata MHH, Sonoda MT, Skaf MS, Elola MD 2009. Anomalous dynamics of hydration water in carbohydrate solutions. J Phys Chem B 113(39):12999-13006.
39. Perticaroli S, Nakanishi M, Pashkovski E, Sokolov AP 2013. Dynamics of hydration water in sugars and peptides solutions. J Phys Chem B 117(25):7729-7736.
40. Winther LR, Qvist J, Halle B 2012. Hydration and mobility of trehalose in aqueous solution. J Phys Chem B 116(30):9196-9207.

41. Comez L, Lupi L, Morresi A, Paolantoni M, Sassi P, Fioretto D 2013. More is different: experimental results on the effect of biomolecules on the dynamics of hydration water. J Phys Chem Lett 4(7):1188-1192.
42. Lerbret A, Affouard F, Bordat P, Hedoux A, Guinet Y, Descamps M 2011. Slowing down of water dynamics in disaccharide aqueous solutions. J Non-Cryst Solids 357(2):695-699.
43. Shikata T, Takahashi R, Satokawa Y 2007. Hydration and dynamic behavior of cyclodextrins in aqueous solution. J Phys Chem B 111(42):12239-12247.
44. Sonoda MT, Skaf MS 2007. Carbohydrate clustering in aqueous solutions and the dynamics of confined water. J Phys Chem B 111(41):11948-11956.
45. Mredha MTI, Roy CK, Rahman MM, Mollah MYA, Susan MABH 2013. An electrochemical approach to study water-D(-)fructose interactions. Electrochim Acta 97:231-237.
46. Giangiacomo R 2006. Study of water-sugar interactions at increasing sugar concentration by NIR spectroscopy. Food Chem 96(3):371-379.
47. Afrin T, Karobi SN, Rahman MM, Mollah MYA, Susan MABH 2013. Water structure modification by sugars and its consequence on micellization behavior of cetyltrimethylammonium bromide in aqueous solution. J Solution Chem 42(7):1488-1499.
48. Lerbret A, Bordat P, Affouard F, Descamps M, Migliardo F 2005. How homogeneous are the trehalose, maltose, and sucrose water solutions? An insight from molecular dynamics simulations. J Phys Chem B 109(21):11046-11057.
49. Heyden M, Bruendermann E, Heugen U, Niehues G, Leitner DM, Havenith M 2008. Long-range influence of carbohydrates on the solvation dynamics of water-answers from terahertz absorption measurements and molecular modeling simulations. J Am Chem Soc 130(17):5773-5779.
50. Paolantoni M, Comez L, Gallina ME, Sassi P, Scarponi F, Fioretto D, Morresi A 2009. Light scattering spectra of water in trehalose aqueous solutions: evidence for two different solvent relaxation processes. J Phys Chem B 113(22):7874-7878.
51. Sapir L, Harries D 2011. Linking trehalose self-association with binary aqueous solution equation of state. J Phys Chem B 115(4):624-634.
52. Rampp M, Buttersack C, Ludemann HD 2000. c,T-dependence of the viscosity and the self-diffusion coefficients in some aqueous carbohydrate solutions. Carbohyd Res 328(4):561-572.
53. Sidebottom DL, Tran TD 2010. Universal patterns of equilibrium cluster growth in aqueous sugars observed by dynamic light scattering. Phys Rev E 82(5).
54. Paul S, Paul S 2015. Molecular insights into the role of aqueous trehalose solution on temperature-induced protein denaturation. J Phys Chem B 119(4):1598-1610.
55. Kaminski K, Kaminska E, Adrjanowicz K, Wojnarowska Z, Wlodarczyk P, Grzybowska K, Dulski M, Wrzalik R, Paluch M 2010. Observation of the dynamics of clusters in D-glucose with the use of dielectric spectroscopy. Phys Chem Chem Phys 12(3):723-730.
56. Nikolajski M, Adams GG, Gillis RB, Besong DT, Rowe AJ, Heinze T, Harding SE 2014. Protein-like fully reversible tetramerisation and super-association of an aminocellulose. Sci Rep 4(3861):1-5.
57. Villiers A 1891. Sur la transformation de la fécule en dextrine par le ferment butyrique. C R Acad Sci CXII:435-437.
58. Crini G 2014. Review: a history of cyclodextrins. Chem Rev 114(21):10940-10975.
59. Loftsson T, Duchêne D 2007. Cyclodextrins and their pharmaceutical applications. Int J Pharm 329(1–2):1-11.
60. Freudenberg K, Blomqvist G, Ewald L, Soff K 1936. Hydrolyse und acetolyse der stärke und der schardinger-dextrine. Berichte d D Chem Gesellschaft 69(6):1258-1266.
61. Kratky O, Porod G 1949. Diffuse small-angle scattering of X-rays in colloid systems. J Colloid Sci 4(1):35-70.

62. Kong R, Shi D, Liu R, Wu C, Ni P, Chen M 2013. Preparation and properties of supramolecular aggregation of dual-sensitive cyclodextrins. Acta Chim Sinica 71(11):1540-1546.
63. Rajendiran N, Venkatesh G, Saravanan J 2014. Supramolecular aggregates formed by sulfadiazine and sulfisomidine inclusion complexes with alpha-and beta-cyclodextrins. Spectrochim Acta A 129:157-162.
64. Fernando Silva O, Mariano Correa N, Silber JJ, de Rossi RH, Fernandez MA 2014. Supramolecular assemblies obtained by mixing different cyclodextrins and AOT or BHDC reverse micelles. Langmuir 30(12):3354-3362.
65. Nag S, Duarte L, Bertrand E, Celton V, Castro M, Choudhary V, Guegan P, Feller J-F 2014. Ultrasensitive QRS made by supramolecular assembly of functionalized cyclodextrins and graphene for the detection of lung cancer VOC biomarkers. J Mater Chem B 2(38):6571-6579.
66. Gramage-Doria R, Armspach D, Matt D 2013. Metallated cavitands (calixarenes, resorcinarenes, cyclodextrins) with internal coordination sites. Coordin Chem Rev 257(3-4):776-816.
67. Xiao Y, Ng S-C, Tan TTY, Wang Y 2012. Recent development of cyclodextrin chiral stationary phases and their applications in chromatography. J Chromatogr A 1269:52-68.
68. West C 2014. Enantioselective separations with supercritical fluids - review. Curr Anal Chem 10(1):99-120.
69. Rocco A, Maruska A, Fanali S 2012. Cyclodextrins as a chiral mobile phase additive in nano-liquid chromatography: comparison of reversed-phase silica monolithic and particulate capillary columns. Anal Bioanal Chem 402(9):2935-2943.
70. Rodriguez-Bonilla P, Manuel Lopez-Nicolas J, Mendez-Cazorla L, Garcia-Carmona F 2011. Development of a reversed phase high performance liquid chromatography method based on the use of cyclodextrins as mobile phase additives to determine pterostilbene in blueberries. J Chromatogr B 879(15-16):1091-1097.
71. Kaceriakova D, Spanik I 2014. Utilization of cyclodextrins in gas chromatography: preparation of capillary columns with cyclodextrin stationary phases. Chem Listy 108(5):462-469.
72. Manuel Lopez-Nicolas J, Rodriguez-Bonilla P, Garcia-Carmona F 2014. Cyclodextrins and antioxidants. Crit Rev Food Sci 54(2):251-276.
73. Medronho B, Valente AJM, Costa P, Romano A 2014. Inclusion complexes of rosmarinic acid and cyclodextrins: stoichiometry, association constants, and antioxidant potential. Colloid Polym Sci 292(4):885-894.
74. Mercader-Ros MT, Lucas-Abellan C, Fortea MI, Gabaldon JA, Nunez-Delicado E 2010. Effect of HP-beta-cyclodextrins complexation on the antioxidant activity of flavonols. Food Chem 118(3):769-773.
75. Celik SE, Ozyurek M, Tufan AN, Guclu K, Apak R 2011. Spectroscopic study and antioxidant properties of the inclusion complexes of rosmarinic acid with natural and derivative cyclodextrins. Spectrochim Acta A 78(5):1615-1624.
76. Ammar HO, El-Nahhas SA, Ghorab MM, Salama AH 2012. Chitosan/cyclodextrin nanoparticles as drug delivery system. J Inc Phenom Macro 72(1-2):127-136.
77. Zhang J, Ma PX 2013. Cyclodextrin-based supramolecular systems for drug delivery: recent progress and future perspective. Adv Drug Deliver Rev 65(9):1215-1233.
78. Machin R, Ramon Isasi J, Velaz I 2012. Beta-cyclodextrin hydrogels as potential drug delivery systems. Carbohyd Polym 87(3):2024-2030.
79. Khodaverdi E, Tekie FSM, Hadizadeh F, Esmaeel H, Mohajeri SA, Tabassi SAS, Zohuri G 2014. Hydrogels composed of cyclodextrin inclusion complexes with PLGA-PEG-PLGA triblock copolymers as drug delivery systems. Aaps Pharmscitech 15(1):177-188.
80. Bazhban M, Nouri M, Mokhtari J 2013. Electrospinning of cyclodextrin functionalized chitosan/PVA nanofibers as a drug delivery system. Chinese J Polym Sci 31(10):1343-1351.

81. Mazzaglia A, Sciortino MT, Kandoth N, Sortino S 2012. Cyclodextrin-based nanoconstructs for photoactivated therapies. J Drug Deliv Sci Tec 22(3):235-242.
82. Szente L, Szejtli J, Kis GL 1998. Spontaneous opalescence of aqueous gamma-cyclodextrin solutions: complex formation or self-aggregation? J Pharm Sci 87(6):778-781.
83. Miyajima K, Mukai T, Nakagaki M, Otagiri M, Uekama K 1986. Activity-coefficients of dimethyl-beta-cyclodextrin in aqueous-solutions. B Chem Soc Jpn 59(2):643-644.
84. Miyajima K, Sawada M, Nakagaki M 1983. Viscosity b-coefficients, apparent molar volumes, and activity-coefficients for alpha-cyclodextrin and gamma-cyclodextrin in aqueous-solutions. B Chem Soc Jpn 56(12):3556-3560.
85. Stezowski JJ, Jogun KH, Eckle E, Bartels K 1978. Dimeric beta-cyclodextrin complexes may mimic membrane diffusion transport. Nature 274(5671):617-619.
86. Gallois-Montbrun D, Le Bas G, Mason SA, Prange T, Lesieur S 2013. A highly hydrated alpha-cyclodextrin/1-undecanol inclusion complex: crystal structure and hydrogen-bond network from high-resolution neutron diffraction at 20K. Acta Crystallogr B 69:214-227.
87. Sicard-Roselli C, Perly B, Le Bas G 2001. The respective benefits of X-ray crystallography and NMR for the structural determination of the inclusion complex between butyl-isothiocyanate and alpha-cyclodextrin. J Incl Phenom Macro 39(3-4):333-337.
88. Mentzafos D, Mavridis IM, Lebas G, Tsoucaris G 1991. Structure of the 4-tert-butylbenzil alcohol beta-cyclodextrin complex - common features in the geometry of beta-cyclodextrin dimeric complexes. Acta Crystallogr B 47:746-757.
89. Saenger WR, Jacob J, Gessler K, Steiner T, Hoffmann D, Sanbe H, Koizumi K, Smith SM, Takaha T 1998. Structures of the common cyclodextrins and their larger analogues - beyond the doughnut. Chem Rev 98(5):1787-1802.
90. Denadai AML, Teixeira KI, Santoro MM, Pimenta AMC, Cortes ME, Sinisterra RD 2007. Supramolecular self-assembly of beta-cyclodextrin: an effective carrier of the antimicrobial agent chlorhexidine. Carbohyd Res 342(15):2286-2296.
91. Shigekawa H, Morozumi T, Komiyama M, Yoshimura M, Kawazu A, Saito Y 1991. Scanning tunneling microscopy on cyclodextrin inclusion complexes. J Vac Sci Technol B 9(2):1189-1192.
92. Nascimento CS, Jr., Anconi CPA, Lopes JF, Dos Santos HF, De Almeida WB 2007. An efficient methodology to study cyclodextrin clusters: application to alpha-CD hydrated monomer, dimer, trimer and tetramer. J Incl Phenom Macro 59(3-4):265-277.
93. Bonnet P, Jaime C, Morin-Allory L 2001. Alpha, beta, and gamma-cyclodextrin dimers. Molecular modeling studies by molecular mechanics and molecular dynamics simulations. J Org Chem 66(3):689-692.
94. Zhang H, Tan T, Feng W, van der Spoel D 2012. Molecular recognition in different environments: beta-cyclodextrin dimer formation in organic solvents. J Phys Chem B 116(42):12684-12693.
95. Terdale SS, Dagade DH, Patil KJ 2006. Thermodynamic studies of molecular interactions in aqueous alpha-cyclodextrin solutions: application of McMillan-Mayer and Kirkwood-Buff theories. J Phys Chem B 110(37):18583-18593.
96. Coleman AW, Nicolis I, Keller N, Dalbiez JP 1992. Aggregation of cyclodextrins - an explanation of the abnormal solubility of beta-cyclodextrin. J Inclus Phenom Mol 13(2):139-143.
97. Cai W, Sun T, Shao X, Chipot C 2008. Can the anomalous aqueous solubility of beta-cyclodextrin be explained by its hydration free energy alone? Phys Chem Chem Phys 10(22):3236-3243.
98. Naidoo KJ, Chen JYJ, Jansson JLM, Widmalm G, Maliniak A 2004. Molecular properties related to the anomalous solubility of beta-cyclodextrin. J Phys Chem B 108(14):4236-4238.
99. Dagade DH, Kolhapurkar RR, Patil KJ 2004. Studies of osmotic coefficients and volumetric behaviour on aqueous solutions of beta-cyclodextrin at 298.15 K. Indian J Chem A 43(10):2073-2080.

100. Loftsson T, Masson M, Sigurdsson HH 2002. Cyclodextrins and drug permeability through semi-permeable cellophane membranes. Int J Pharm 232(1-2):35-43.
101. Loftsson T, Magnusdottir A, Masson M, Sigurjonsdottir JF 2002. Self-association and cyclodextrin solubilization of drugs. J Pharm Sci 91(11):2307-2316.
102. Loftsson T, Masson M, Brewster ME 2004. Self-association of cyclodextrins and cyclodextrin complexes. J Pharm Sci 93(5):1091-1099.
103. Sigurdsson HH, Magnusdottir A, Masson M, Loftsson T 2002. The effects of cyclodextrins on hydrocortisone permeability through semi-permeable membranes. J Incl Phenom Macro 44(1-4):163-167.
104. Magnusdottir A, Masson M, Loftsson T 2002. Self-association and cyclodextrin solubilization of NSAIDs. J Incl Phenom Macro 44(1-4):213-218.
105. Messner M, Kurkov SV, Jansook P, Loftsson T 2010. Self-assembled cyclodextrin aggregates and nanoparticles. Int J Pharm 387(1-2):199-208.
106. He Y, Fu P, Shen X, Gao H 2008. Cyclodextrin-based aggregates and characterization by microscopy. Micron 39(5):495-516.
107. Gonzalez-Gaitano G, Rodriguez P, Isasi JR, Fuentes M, Tardajos G, Sanchez M 2002. The aggregation of cyclodextrins as studied by photon correlation spectroscopy. J Incl Phenom Macro 44(1-4):101-105.
108. Puskas I, Schrott M, Malanga M, Szente L 2013. Characterization and control of the aggregation behavior of cyclodextrins. J Incl Phenom Macro 75(3-4):269-276.
109. Bonini M, Rossi S, Karlsson G, Almgren M, Lo Nostro P, Baglioni P 2006. Self-assembly of beta-cyclodextrin in water. Part 1: Cryo-TEM and dynamic and static light scattering. Langmuir 22(4):1478-1484.
110. Wu AH, Shen XH, He YK 2006. Investigation on gamma-cyclodextrin nanotube induced by N,N '-diphenylbenzidine molecule. J Colloid Interf Sci 297(2):525-533.
111. Katritzky AR, Pacureanu LM, Slavov SH, Dobchev DA, Karelson M 2008. QSPR study of critical micelle concentrations of nonionic surfactants. Ind Eng Chem Res 47(23):9687-9695.
112. Dominguez A, Fernandez A, Gonzalez N, Iglesias E, Montenegro L 1997. Determination of critical micelle concentration of some surfactants by three techniques. J Chem Educ 74(10):1227-1231.
113. Ruso JM, Attwood D, Taboada P, Mosquera V, Sarmiento F 2000. Light scattering and NMR studies on the self-aggregation of sodium n-hexyl sulfate in aqueous electrolyte solution. Langmuir 16(4):1620-1625.
114. Singh T, Kumar A 2007. Aggregation behavior of ionic liquids in aqueous solutions: effect of alkyl chain length, cations, and anions. J Phys Chem B 111(27):7843-7851.
115. Bowers J, Butts CP, Martin PJ, Vergara-Gutierrez MC, Heenan RK 2004. Aggregation behavior of aqueous solutions of ionic liquids. Langmuir 20(6):2191-2198.
116. Singh T, Kumar A 2008. Self-aggregation of ionic liquids in aqueous media: a thermodynamic study. Colloids Surf A 318(1-3):263-268.
117. Garofalakis G, Murray BS, Sarney DB 2000. Surface activity and critical aggregation concentration of pure sugar esters with different sugar headgroups. J Colloid Interf Sci 229(2):391-398.
118. De Sousa FB, Lima AC, Denadai AML, Anconi CPA, De Almeida WB, Novato WTG, Dos Santos HF, Drum CL, Langer R, Sinisterra RD 2012. Superstructure based on beta-CD self-assembly induced by a small guest molecule. Phys Chem Chem Phys 14(6):1934-1944.
119. Garnero C, Longhi M 2007. Study of ascorbic acid interaction with hydroxypropyl-beta-cyclodextrin and triethanolamine, separately and in combination. J Pharm Biomed Anal 45(4):536-545.
120. Garnero C, Zoppi A, Genovese D, Longhi M 2010. Studies on trimethoprim:hydroxypropyl-beta-cyclodextrin: aggregate and complex formation. Carbohyd Res 345(17):2550-2556.

121. Palma S, Manzo RH, Allemandi D, Fratoni L, Lo Nostro P 2003. Drugs solubilization in ascorbyl-decanoate micellar solutions. Colloids Surf A 212(2-3):163-173.
122. Szejtli J. 1988. Cyclodextrins. Cyclodextrin Technology, ed.: Springer Netherlands. p 1-78.
123. Loftsson T 2014. Self-assembled cyclodextrin nanoparticles and drug delivery. J Incl Phenom Macro 80(1-2):1-7.
124. Rao JP, Geckeler KE 2011. Cyclodextrin supramacromolecules: unexpected formation in aqueous phase under ambient conditions. Macromol Rapid Comm 32(5):426-430.
125. Geze A, Putaux JL, Choisnard L, Jehan P, Wouessidjewe D 2004. Long-term shelf stability of amphiphilic beta-cyclodextrin nanosphere suspensions monitored by dynamic light scattering and cryo-transmission electron microscopy. J Microencapsul 21(6):607-613.
126. Polarz S, Smarsly B, Bronstein L, Antonietti M 2001. From cyclodextrin assemblies to porous materials by silica templating. Angew Chem Int Ed 40(23):4417-4421.
127. Hunt MA, Rusa CC, Tonelli AE, Balik CM 2004. Structure and stability of columnar cyclomaltohexaose (alpha-cyclodextrin) hydrate. Carbohyd Res 339(17):2805-2810.
128. Hunt MA, Rusa CC, Tonelli AE, Balik CM 2005. Structure and stability of columnar cyclomaltooctaose (gamma-cyclodextrin) hydrate. Carbohyd Res 340(9):1631-1637.
129. Liu L, Guo QX 1999. Novel prediction for the driving force and guest orientation in the complexation of alpha- and beta-cyclodextrin with benzene derivatives. J Phys Chem B 103(17):3461-3467.
130. Brewster ME, Loftsson T 2007. Cyclodextrins as pharmaccutical solubilizers. Adv Drug Deliver Rev 59(7):645-666.
131. Alvira E, Garcia J, Mayoral J 2000. Factors influencing beta-cyclodextrin inclusion complex formation. Recent Res Dev Chem Phys 1:89-100.
132. Gabelica V, Galic N, De Pauw E 2002. On the specificity of cyclodextrin complexes detected by electrospray mass spectrometry. J Am Soc Mass Spectr 13(8):946-953.
133. Morrison PWJ, Connon CJ, Khutoryanskiy VV 2013. Cyclodextrin-mediated enhancement of riboflavin solubility and corneal permeability. Mol Pharm 10(2):756-762.
134. Dahan A, Miller JM, Hoffman A, Amidon GE, Amidon GL 2010. The solubility-permeability interplay in using cyclodextrins as pharmaceutical solubilizers: mechanistic modeling and application to progesterone. J Pharm Sci 99(6):2739-2749.
135. Pradines B, Gallard J-F, Iorga BI, Gueutin C, Loiseau PM, Ponchel G, Bouchemal K 2014. Investigation of the complexation of albendazole with cyclodextrins for the design of new antiparasitic formulations. Carbohyd Res 398:50-55.
136. Rawat S, Jain SK 2003. Rofecoxib-beta-cyclodextrin inclusion complex for solubility enhancement. Pharmazie 58(9):639-641.
137. K SK, M S, Y PR 2013. Dissolution enhancement of poorly soluble drugs by using complexation technique – a review. J Pharm Sci & Res 5(5):120-124.
138. Wen XH, Tan F, Jing ZJ, Liu ZY 2004. Preparation and study the 1 : 2 inclusion complex of carvedilol with beta-cyclodextrin. J Pharmaceut Biomed 34(3):517-523.
139. Loftsson T, Brewster ME 2011. Pharmaceutical applications of cyclodextrins: effects on drug permeation through biological membranes. J Pharm Pharmacol 63(9):1119-1135.
140. Kratz JM, Teixeira MR, Ferronato K, Teixeira HF, Koester LS, Simoes CMO 2012. Preparation, characterization, and in vitro intestinal permeability evaluation of thalidomide-hydroxypropyl-beta-cyclodextrin complexes. Aaps Pharmscitech 13(1):118-124.
141. Ge X, Huang Z, Tian S, Huang Y, Zeng C 2012. Complexation of carbendazim with hydroxypropyl-beta-cyclodextrin to improve solubility and fungicidal activity. Carbohyd Polym 89(1):208-212.
142. Anwer MK, Jamil S, Ansari MJ, Al-Shdefat R, Ali BE, Ganaie MA, Abdel-Kader MS, Shakeel F 2014. Water soluble binary and ternary complexes of diosmin with beta-cyclodextrin: spectroscopic characterization, release studies and anti-oxidant activity. J Mol Liq 199:35-41.

143. Dandawate PR, Vyas A, Ahmad A, Banerjee S, Deshpande J, Swamy KV, Jamadar A, Dumhe-Klaire AC, Padhye S, Sarkar FH 2012. Inclusion complex of novel curcumin analogue CDF and beta-cyclodextrin (1:2) and its enhanced in vivo anticancer activity against pancreatic cancer. Pharm Res 29(7):1775-1786.
144. Venuti V, Cannava C, Cristiano MC, Fresta M, Majolino D, Paolino D, Stancanelli R, Tommasini S, Ventura CA 2014. A characterization study of resveratrol/sulfobutyl ether-beta-cyclodextrin inclusion complex and in vitro anticancer activity. Colloid Surface B 115:22-28.
145. Zhang H, Chen M, He Z, Wang Z, Zhang M, He Z, Wan Q, Liang D, Repka MA, Wu C 2013. Molecular modeling-based inclusion mechanism and stability studies of doxycycline and hydroxypropyl-beta-cyclodextrin complex for ophthalmic delivery. Aaps Pharmscitech 14(1):10-18.
146. Tien An N, Liu B, Zhao J, Thomas DS, Hook JM 2013. An investigation into the supramolecular structure, solubility, stability and antioxidant activity of rutin/cyclodextrin inclusion complex. Food Chem 136(1):186-192.
147. Yuan C, Du L, Jin Z, Xu X 2013. Storage stability and antioxidant activity of complex of astaxanthin with hydroxypropyl-beta-cyclodextrin. Carbohyd Polym 91(1):385-389.
148. Kurkov SV, Loftsson T 2013. Cyclodextrins. Int J Pharm 453(1):167-180.
149. Ferrazza R, Rossi B, Guella G 2014. DOSY-NMR and Raman investigations on the self-aggregation and cyclodextrin complexation of vanillin. J Phys Chem B 118(25):7147-7155.
150. Nogueiras-Nieto L, Sobarzo-Sanchez E, Luis Gomez-Amoza J, Otero-Espinar FJ 2012. Competitive displacement of drugs from cyclodextrin inclusion complex by polypseudorotaxane formation with poloxamer: implications in drug solubilization and delivery. Eur J Pharm Biopharm 80(3):585-595.
151. De Sousa FB, Leite Denadai AM, Lula IS, Nascimento CS, Jr., Fernandes Neto NSG, Lima AC, De Almeida WB, Sinisterra RD 2008. Supramolecular self-assembly of cyclodextrin and higher water soluble guest: thermodynamics and topological studies. J Am Chem Soc 130(26):8426-8436.
152. Correia I, Bezzenine N, Ronzani N, Platzer N, Beloeil JC, Doan BT 2002. Study of inclusion complexes of acridine with beta- and (2,6-di-O-methyl)-beta-cyclodextrin by use of solubility diagrams and NMR spectroscopy. J Phys Org Chem 15(9):647-659.
153. Ridhurkar DN, Ansari KA, Kumar D, Kaul NS, Krishnamurthy T, Dhawan S, Pillai R 2013. Inclusion complex of aprepitant with cyclodextrin: evaluation of physico-chemical and pharmacokinetic properties. Drug Dev Ind Pharm 39(11):1783-1792.
154. Andronati SA, Shapiro YE, Yakubovskaya LN, Gorbatyuk VY, Andronati KS, Krasnoschekaya SP 1996. Inclusion compounds of psychotropic agents and cyclodextrins. J Inclus Phenom Mol 24(1-2):175-186.
155. Nobusawa K, Payra D, Naito M 2014. Pyridyl-cyclodextrin for ultra-hydrosolubilization of 60 fullerene. Chem Commun 50(61):8339-8342.
156. Loftsson T, Brewster ME 2012. Cyclodextrins as functional excipients: methods to enhance complexation efficiency. J Pharm Sci 101(9):3019-3032.
157. Messner M, Kurkov SV, Flavia-Piera R, Brewster ME, Loftsson T 2011. Self-assembly of cyclodextrins: The effect of the guest molecule. Int J Pharm 408(1-2):235-247.
158. Liptaj T, Pronayova N, Kralova K 1995. NMR-study of the inclusion complex of cationic surfactant n,n'-bis 4-(4-tert-butylphenoxy)butyldimethyl -1,6-hexanediammonium dibromide with beta-cyclodextrin. Pharmazie 50(11):744-747.
159. Mele A, Mendichi R, Selva A 1998. Non-covalent associations of cyclomaltooligosaccharides (cyclodextrins) with trans-beta-carotene in water: evidence for the formation of large aggregates by light scattering and NMR spectroscopy. Carbohyd Res 310(4):261-267.
160. Fülöp Z, Saokham P, Loftsson T 2014. Sulfobutylether-beta-cyclodextrin/chitosan nano- and microparticles and their physicochemical characteristics. Int J Pharm 472(1-2):282-287.

161. Jansook P, Kurkov SV, Loftsson T 2010. Cyclodextrins as solubilizers: formation of complex aggregates. J Pharm Sci 99(2):719-729.
162. Saokham P, Loftsson T 2015. A new approach for quantitative determination of γ-cyclodextrin in aqueous solutions: application in aggregate determinations and solubility in hydrocortisone/γ-cyclodextrin inclusion complex. J Pharm Sci in press.
163. Jansook P, Moya-Ortega MD, Loftsson T 2010. Effect of self-aggregation of gamma-cyclodextrin on drug solubilization. J Incl Phenom Macro 68(1-2):229-236.
164. Muankaew C, Jansook P, Stefansson E, Loftsson T 2014. Effect of gamma-cyclodextrin on solubilization and complexation of irbesartan: influence of pH and excipients. Int J Pharm 474(1-2):80-90.
165. Loftsson T, Vogensen SB, Desbos C, Jansook P 2008. Carvedilol: solubilization and cyclodextrin complexation: a technical note. Aaps Pharmscitech 9(2):425-430.
166. Moya-Ortega MD, Alves TFG, Alvarez-Lorenzo C, Concheiro A, Stefansson E, Thorsteinsdottir M, Loftsson T 2013. Dexamethasone eye drops containing gamma-cyclodextrin-based nanogels. Int J Pharm 441(1-2):507-515.
167. Jansook P, Stefansson E, Thorsteinsdottir M, Sigurdsson BB, Kristjansdottir SS, Bas JF, Sigurdsson HH, Loftsson T 2010. Cyclodextrin solubilization of carbonic anhydrase inhibitor drugs: Formulation of dorzolamide eye drop microparticle suspension. Eur J Pharm Biopharm 76(2):208-214.
168. Auzely-Velty R, Perly B, Tache O, Zemb T, Jehan P, Guenot P, Dalbiez JP, Djedaini-Pilard F 1999. Cholesteryl-cyclodextrins: synthesis and insertion into phospholipid membranes. Carbohyd Res 318(1-4):82-90.
169. Auzely-Velty R, Djedaini-Pilard F, Desert S, Perly B, Zemb T 2000. Micellization of hydrophobically modified cyclodextrins. 1. Micellar structure. Langmuir 16(8):3727-3734.
170. Gervaise C, Bonnet V, Wattraint O, Aubry F, Sarazin C, Jaffres P-A, Djedaini-Pilard F 2012. Synthesis of lipophosphoramidyl-cyclodextrins and their supramolecular properties. Biochimie 94(1):66-74.
171. Klaus A, Fajolles C, Bauer M, Collot M, Mallet J-M, Daillant J 2011. Amphiphilic behavior and membrane solubility of a dicholesteryl-cyclodextrin. Langmuir 27(12):7580-7586.
172. Skiba M, Nemati F, Puisieux F, Duchene D, Wouessidjewe D 1996. Spontaneous formation of drug-containing amphiphilic beta-cyclodextrin nanocapsules. Int J Pharm 145(1-2):241-245.
173. Fendler JH 1996. Self-assembled nanostructured materials. Chem Mater 8(8):1616-1624.
174. Attwood D, Florence A. 1983. Surfactant systems: their chemistry, pharmacy and biology. ed., London EC4P 4EE, United Kingdom: Chapman & Hall. p 794.
175. Malmsten M. 2002. Surfactants and polymers in drug delivery. ed., New York: Marcel Dekker. p 348.
176. Kronberg B, Holmberg K, Lindman B. 2014. Surface chemistry of surfactants and polymers. ed., West Sussex, UK: John Wiley & Sons, Ltd. p 479.
177. Loftsson T, Frioriksdottir H, Sigurdardottir AM, Ueda H 1994. The effect of water-soluble polymers on drug-cyclodextrin complexation. Int J Pharm 110(2):169-177.
178. Loftsson T, Frioriksdottir H 1998. The effect of water-soluble polymers on the aqueous solubility and complexing abilities of beta-cyclodextrin. Int J Pharm 163(1-2):115-121.
179. Loftsson T, Matthiasson K, Masson M 2003. The effects of organic salts on the cyclodextrin solubilization of drugs. Int J Pharm 262(1-2):101-107.
180. Loftsson T, Masson M 2004. The effects of water-soluble polymers on cyclodextrins and cyclodextrin solubilization of drugs. J Drug Deliv Sci Tec 14(1):35-43.
181. Fenyvesi E, Vikmon M, Szeman J, Redenti E, Delcanale M, Ventura P, Szejtli J 1999. Interaction of hydroxy acids with β-cyclodextrin. J Incl Phenom Macro 33(3):339-344.
182. Redenti E, Szente L, Szejtli J 2000. Drug/cyclodextrin/hydroxy acid multicomponent systems. Properties and pharmaceutical applications. J Pharm Sci 89(1):1-8.

183. Redenti E, Szente L, Szejtli J 2001. Cyclodextrin complexes of salts of acidic drugs. Thermodynamic properties, structural features, and pharmaceutical applications. J Pharm Sci 90(8):979-986.
184. Mura P, Maestrelli F, Cirri M 2003. Ternary systems of naproxen with hydroxypropyl-beta-cyclodextrin and aminoacids. Int J Pharm 260(2):293-302.
185. Jansook P, Loftsson T 2009. CDs as solubilizers: effects of excipients and competing drugs. Int J Pharm 379(1):32-40.
186. Zia V, Rajewski RA, Stella VJ 2000. Thermodynamics of binding of neutral molecules to sulfobutyl ether beta-cyclodextrins (SBE-beta-CDs): the effect of total degree of substitution. Pharm Res 17(8):936-941.
187. Mennini N, Bragagni M, Maestrelli F, Mura P 2014. Physico-chemical characterization in solution and in the solid state of clonazepam complexes with native and chemically-modified cyclodextrins. J Pharm Biomed Anal 89:142-149.
188. Puskás I, Czifra TC, Fenyvesi É, Szente L 2013. Aggregation behavior of cyclodextrin and cholesterol in simulated human cerebrospinal fluid. Bioact Carbohydr Dietary Fibre 2(2):157-163.
189. Takahashi AI, Veiga BFJ, Ferraz HG 2012. A literature review of cyclodextrin inclusion complexes characterization. Part III. Differential scanning calorimetry and thermogravimetry. Int J Pharm Sci Rev Res 12(1):16-20.
190. Takahashi AI, Viega BFJ, Ferraz HG 2012. A literature review of yclodextrin inclusion complexes characterization. Part II. X-ray diffraction, infrared spectroscopy and nuclear magnetic resonance. Int J Pharm Sci Rev Res 12(1):8.
191. Bersier PM, Bersier J, Klingert B 1991. Electrochemistry of cyclodextrins and cyclodextrin inclusion complexes. Electroanal 3(6):443-455.
192. Mura P 2014. Analytical techniques for characterization of cyclodextrin complexes in aqueous solution: a review. J Pharmaceut Biomed 101:238-250.
193. Pessine FBT, Calderini A, Alexandrino GL. 2012. Review: cyclodextrin inclusion complexes probed by NMR techniques. In Kim D-H, editor Magnetic Resonance Spectroscopy, ed., China: INTECH. p 237-264.
194. Singh R, Bharti N, Madan J, Hiremath SN 2010. Characterization of cyclodextrin inclusion complexes - a review. J Pharm Sci Technol 2(3):171-183.
195. Lebrilla CB 2001. The gas-phase chemistry of cyclodextrin inclusion complexes. Acc Chem Res 34(8):653-661.
196. Kralj B, Smidovnik A, Kobe J 2009. Mass spectrometric investigations of alpha- and beta-cyclodextrin complexes with ortho-, meta- and para-coumaric acids by negative mode electrospray ionization. Rapid Commun Mass Spectrom 23(1):171-180.
197. Ivanova B, Spiteller M 2014. Macromolecular ensembles of cyclodextrin crystallohydrates and clathrates - experimental and theoretical gas - and condense phase study. Int J Biol Macromol 64:383-391.
198. Zimnicka M, Troc A, Ceborska M, Jakubczak M, Kolinski M, Danikiewicz W 2014. Structural elucidation of specific noncovalent association of folic acid with native cyclodextrins using an ion mobility mass spectrometry and theoretical approach. Anal Chem 86(9):4249-4255.
199. Loftsson T. 2015. Essential pharmacokinetics: a primer for pharmaceutical scientists. ed.: Elsevier Science. p 178.
200. Loftsson T 2015. Excipient pharmacokinetics and profiling. Int J Pharm 480(1-2):48-54.
201. Tanito M, Hara K, Takai Y, Matsuoka Y, Nishimura N, Jansook P, Loftsson T, Stefansson E, Ohira A 2011. Topical dexamethasone-cyclodextrin microparticle eye drops for diabetic macular edema. Invest Ophth Vis Sci 52(11):7944-7948.
202. Konradsdottir F, Ogmundsdottir H, Sigurdsson V, Loftsson T 2009. Drug targeting to the hair follicles: a cyclodextrin-based drug delivery. Aaps Pharmscitech 10(1):266-269.

203. Jóhannsdóttir S, Jansook P, Stefánsson E, Loftsson T 2015. Development of a cyclodextrin-based aqueous cyclosporin A eye drop formulation. Int J Pharm 493:86-95.
204. Ohira A, Tanito M, Jóhannesson G, Ásgrímsdóttir GM, Lund SH, Loftsson T, Stefánsson E 2015. Topical dexamethasone γ-cyclodextrin nanoparticle eye drops increase visual acuity and decrease macular thickness in diabetic macular edema; a prospective randomized controlled clinical trial. Acta Ophthalmol:in print.
205. Messner M, Kurkov SV, Palazon MM, Fernandez BA, Brewster ME, Loftsson T 2011. Self-assembly of cyclodextrin complexes: effect of temperature, agitation and media composition on aggregation. Int J Pharm 419(1-2):322-328.
206. Kostewicz ES, Abrahamsson B, Brewster M, Brouwers J, Butler J, Carlert S, Dickinson PA, Dressman J, Holm R, Klein S, Mann J, McAllister M, Minekus M, Muenster U, Mullertz A, Verwei M, Vertzoni M, Weitschies W, Augustijns P 2014. In vitro models for the prediction of in vivo performance of oral dosage forms. Eur J Pharm Sci 57:342-366.
207. McAllister M 2010. Dynamic dissolution: a step closer to predictive dissolution testing? Mol Pharm 7(5):1374-1387.
208. Fülöp Z, Nielsen TT, Larsen KL, Loftsson T 2013. Dextran-based cyclodextrin polymers: their solubilizing effect and self-association. Carbohyd Polym 97(2):635-642.
209. Brouwers J, Augustijns P 2014. Resolving intraluminal drug and formulation behavior: gastrointestinal concentration profiling in humans. Eur J Pharm Sci 61:2-10.
210. Hens B, Brouwers J, Anneveld B, Corsetti M, Symillides M, Vertzoni M, Reppas C, Turner DB, Augustijns P 2014. Gastrointestinal transfer: in vivo evaluation and implementation in in vitro and in silico predictive tools. Eur J Pharm Sci 63:233-242.
211. Weitschies W, Wilson CG 2011. In vivo imaging of drug delivery systems in the gastrointestinal tract. Intl J Pharm 417(1-2):216-226.
212. Galia E, Nicolaides E, Horter D, Lobenberg R, Reppas C, Dressman JB 1998. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. Pharm Res 15(5):698-705.
213. Furune T, Ikuta N, Ishida Y, Okamoto H, Nakata D, Terao K, Sakamoto N 2014. A study on the inhibitory mechanism for cholesterol absorption by alpha-cyclodextrin administration. Beilstein J Org Chem 10:2827-2835.
214. Holm R, Nicolajsen HV, Hartvig RA, Westh P, Ostergaard J 2007. Complexation of tauro- and glyco-conjugated bile salts with three neutral beta-CDs studied by ACE. Electrophoresis 28(20):3745-3752.
215. Holm R, Schonbeck C, Askjaer S, Jensen H, Westh P, Ostergaard J 2011. Complexation of tauro- and glyco-conjugated bile salts with α-cyclodextrin and hydroxypropyl-α-cyclodextrin studied by affinity capillary electrophoresis and molecular modelling. J Sep Sci 34(22):3221-3230.
216. Rajabi K, Ashcroft AE, Radford SE 2015. Mass spectrometric methods to analyze the structural organization of macromolecular complexes. Methods 89:13-21.
217. Mehmood S, Allison TM, Robinson CV. 2015. Mass spectrometry of protein complexes: from origins to applications. Annual Review of Physical Chemistry, Vol 66, ed., Palo Alto: Annual Reviews. p 453-474.
218. Loftsson T 2010. Aqueous solubility and true solutions. Pharmazie 65(6):404-407.
219. Loftsson T, Masson M, Sigurjonsdottir JF 1999. Methods to enhance the complexation efficiency of cyclodextrins. Stp Pharma Sci 9(3):237-242.
220. Sabadini E, Cosgrove T, Egidio FC 2006. Solubility of cyclomaltooligosaccharides (cyclodextrins) in H2O and D2O: a comparative study. Carbohyd Res 341(2):270-274.

Figure 1. A: Simple amphiphiles such as fatty acids and alcohols form micelles in aqueous solutions. The micelles have a lipophilic core and a hydrophilic surface. Formation of micelles minimizes the contact surface between water molecules and the non-polar group of an amphiphile with a consequent decrease in free energy. B: Hydrophobic drugs such as steroids do also self-assemble in aqueous solutions to form dimers, trimers and higher order oligomers to reduce their contact surface with water.[218]

Figure 2. A fragment of hydrogen bond network of liquid water. Hydrogen bonds marked by black dotted line.

Figure 3. Number of water molecules in the hydration shell of glucose molecules that are shared with a second solute molecule. Based on work Fioretto et al.[36]

Figure 4. The effect of solute concentration on the viscosity of pure aqueous 2-hydroxypropyl-β-CD (HPβCD) and glucose solutions. Based on[219] and data from Nordic Sugar A/S, Denmark (www.nordicsugar.com).

Figure 5. Normalized mean trehalose cluster size for at different trehalose concentration in pure water where n_{tre} corresponds to the weighted average number of trehalose molecules in a given cluster and N_{tre} to the total number of trehalose molecules in a given system. Based on molecular dynamic simulations.[54]

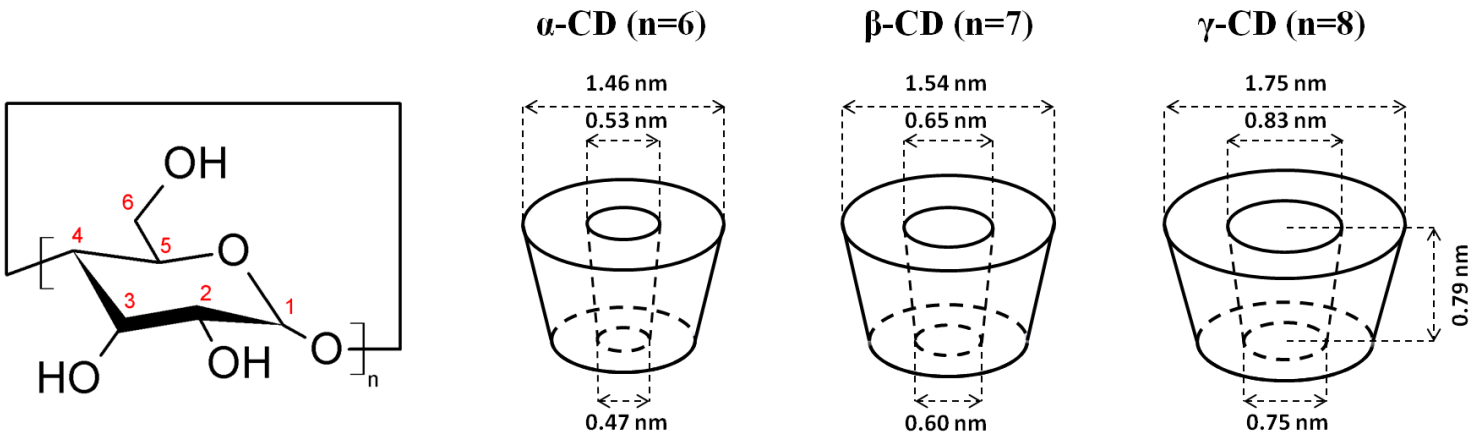
Figure 6. An average size of native CD aggregates (n is the number of molecules) versus CD concentration observed by light scattering taken from literature. Reprinted from[105].

Figure 7. Schematic representation of the packing of CD molecules in the cage (a) and channel (b) crystal structures.

Figure 8. Schematic representation of a drug-CD system in aqueous solution.

Figure 9. Formation of complex aggregates in aqueous solution.

Table 1. Structure and physicochemical properties of the natural αCD, βCD, and γCD and some of their derivatives.



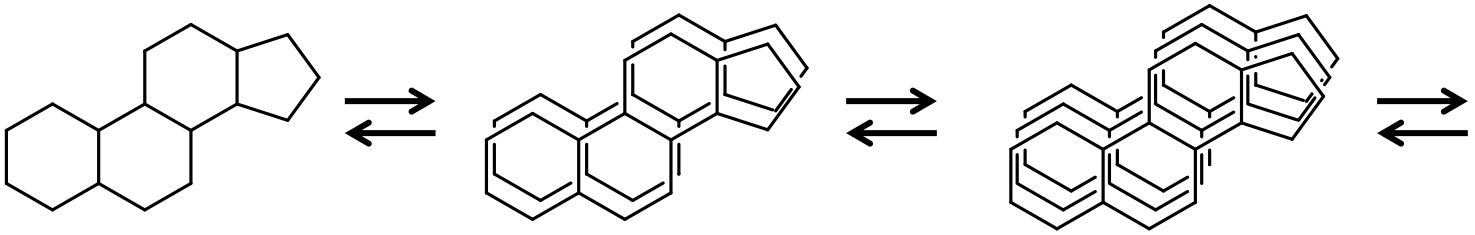
Properties	αCD	βCD	HPβCD	SBEβCD	RMβCD	γCD	HPγCD
Full name:	α-CD	β-CD	2-Hydroxypropyl-βCD	Sulfobutyl ether βCD sodium salt	Randomly methylated βCD	γ-CD	2-Hydroxypropyl-γCD
Molar substitution:	-	-	0.65	0.9	1.8	-	0.6
Molecular weight of anhydrous compound (Da):	972.8	1135	1400	2163	1312	1297	1576
Calculated $LogP_{(octanol/water)}$ at 25°C:[a]	-13	-14	-11	< -10	-6	-17	-16
Approx. solubility in water at 25°C (mg/ml):[a, b]	130	18.4	> 600	> 500	> 600	250	> 600
Number of H-donors:	18	21	21	15	8	24	24
Number of H-acceptors:	30	35	39	53	35	40	45

[a] From SciFinder, ACS, USA (scifinder.cas.org).
[b] From work of Sabadini et al.[220]

Table 2. Formation and stability of CD aggregates in aqueous solutions.

Property	
The natural CDs	The natural αCD, βCD and γCD do form aggregates by themselves in pure aqueous CD solutions but the aggregation is very low with much less than 1 % of dissolved CD present in the form of aggregates.
Formation of inclusion complexes	Formation of drug/CD inclusion complexes increases the aggregation, both in the case of the natural CDs and in the case of the randomly substituted CD derivatives.
The aggregate diameter	The diameter of the complex aggregates depends on the drug/CD properties as well as the concentration of drug/CD inclusion complexes, increasing with increasing availability of drug/CD complexes.
The degree of aggregation	The degree of aggregation depends on the availability of drug/CD complexes in the aqueous solution, increasing with increasing availability of drug/CD complexes.
Effects of organic solvents and temperature	Addition of organic solvents such as ethanol that are known to decrease the complexation will reduce the aggregation. Also, heating of aqueous CD solutions will result in decreased aggregation.
Dynamic equilibrium	In aqueous solutions drug/CD complex aggregates are in dynamic equilibrium with un-aggregated (i.e., free) complexes. Aggregates are constantly being formed and dissembled.
The aggregates are unstable	The complex aggregates are unstable and dissemble up on media dilution.
Limited solubility	Complexes of the natural αCD, βCD and γCD have limited solubility in water and tend to precipitate in aqueous solutions as solid drug/CD complex aggregates. Their complexes are frequently less soluble than the CDs themselves.

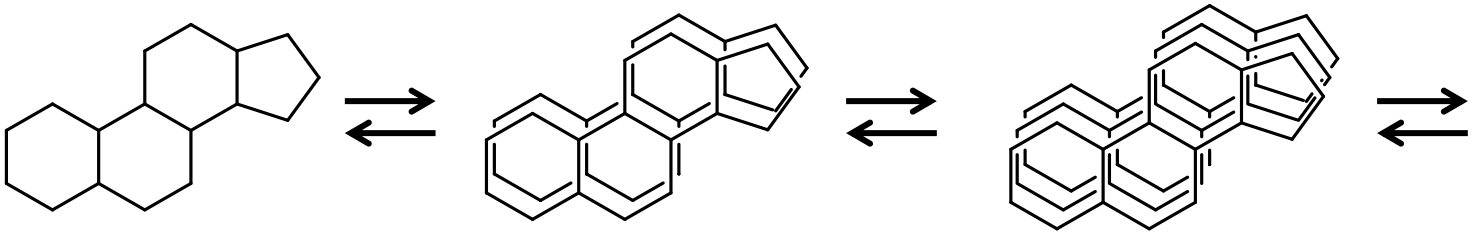
B



Monomer Dimer Trimer

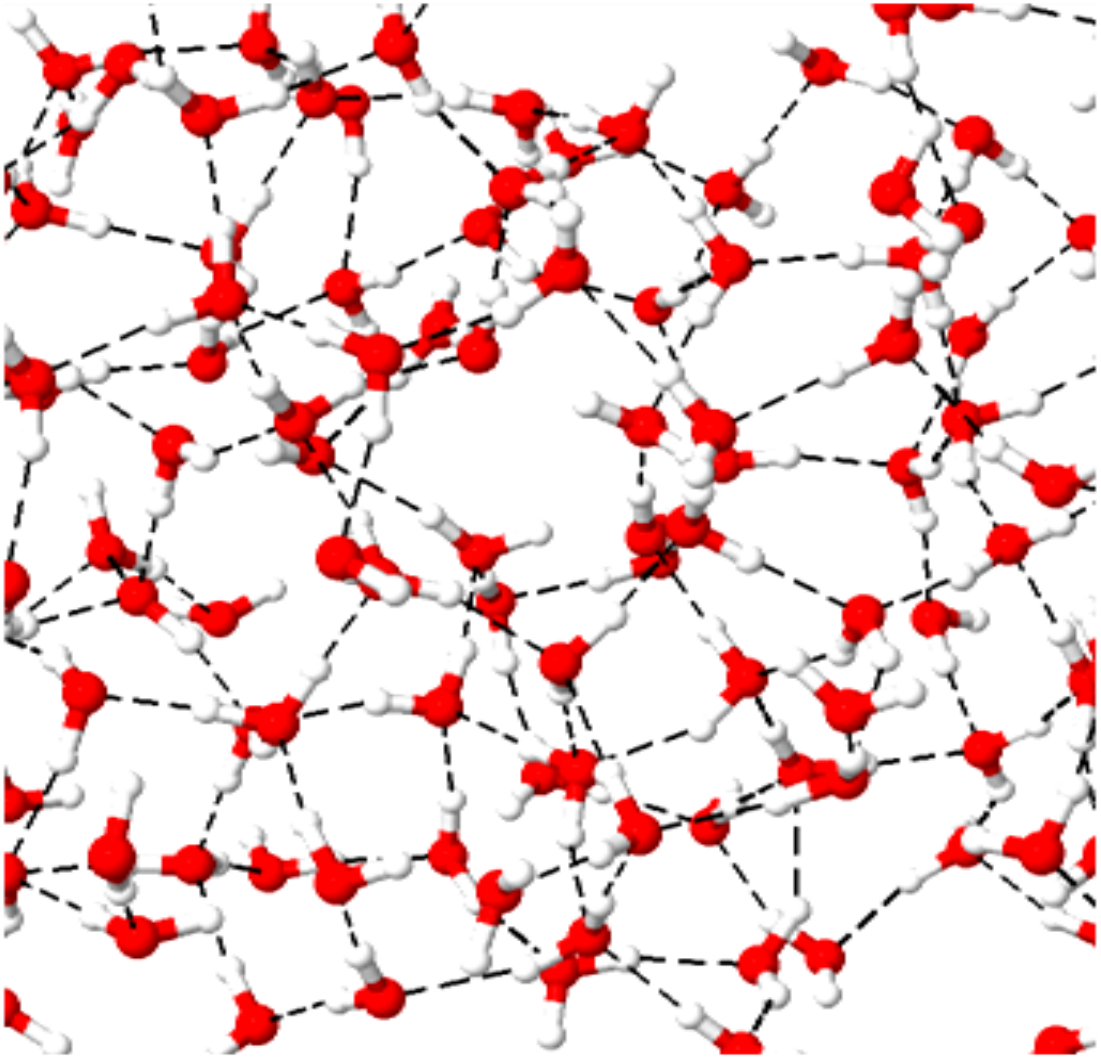
Higher order oligomers

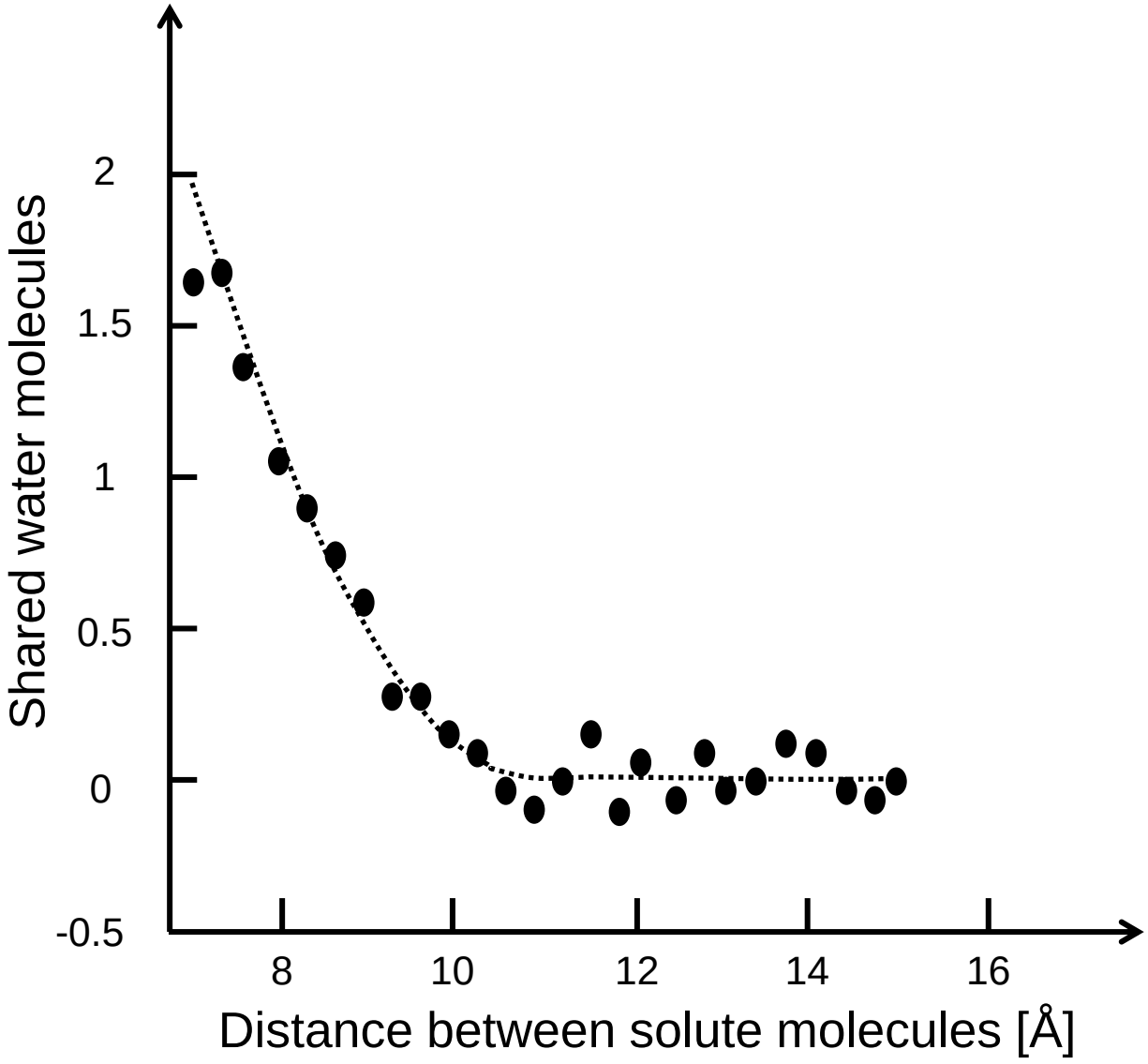
B

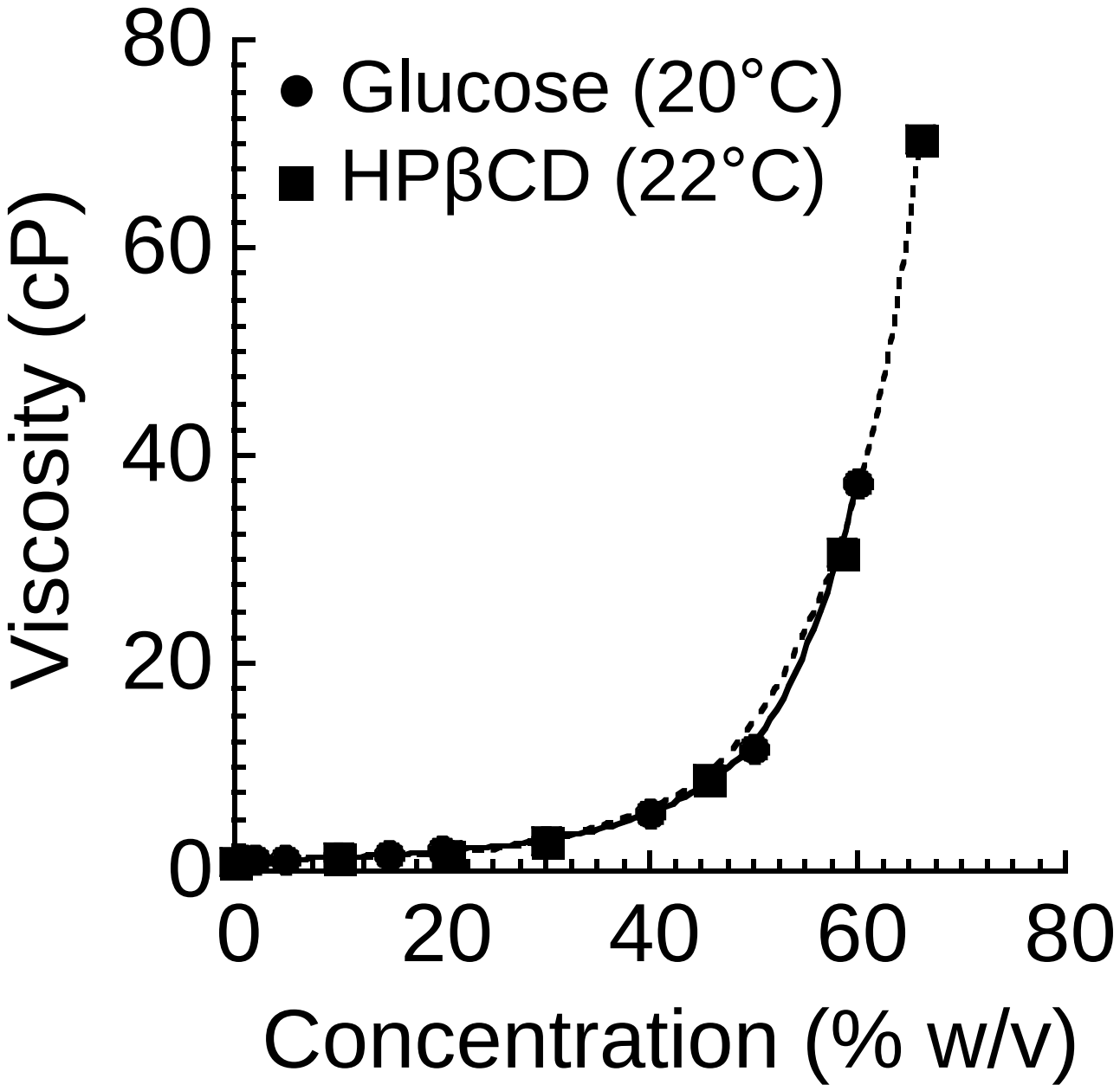


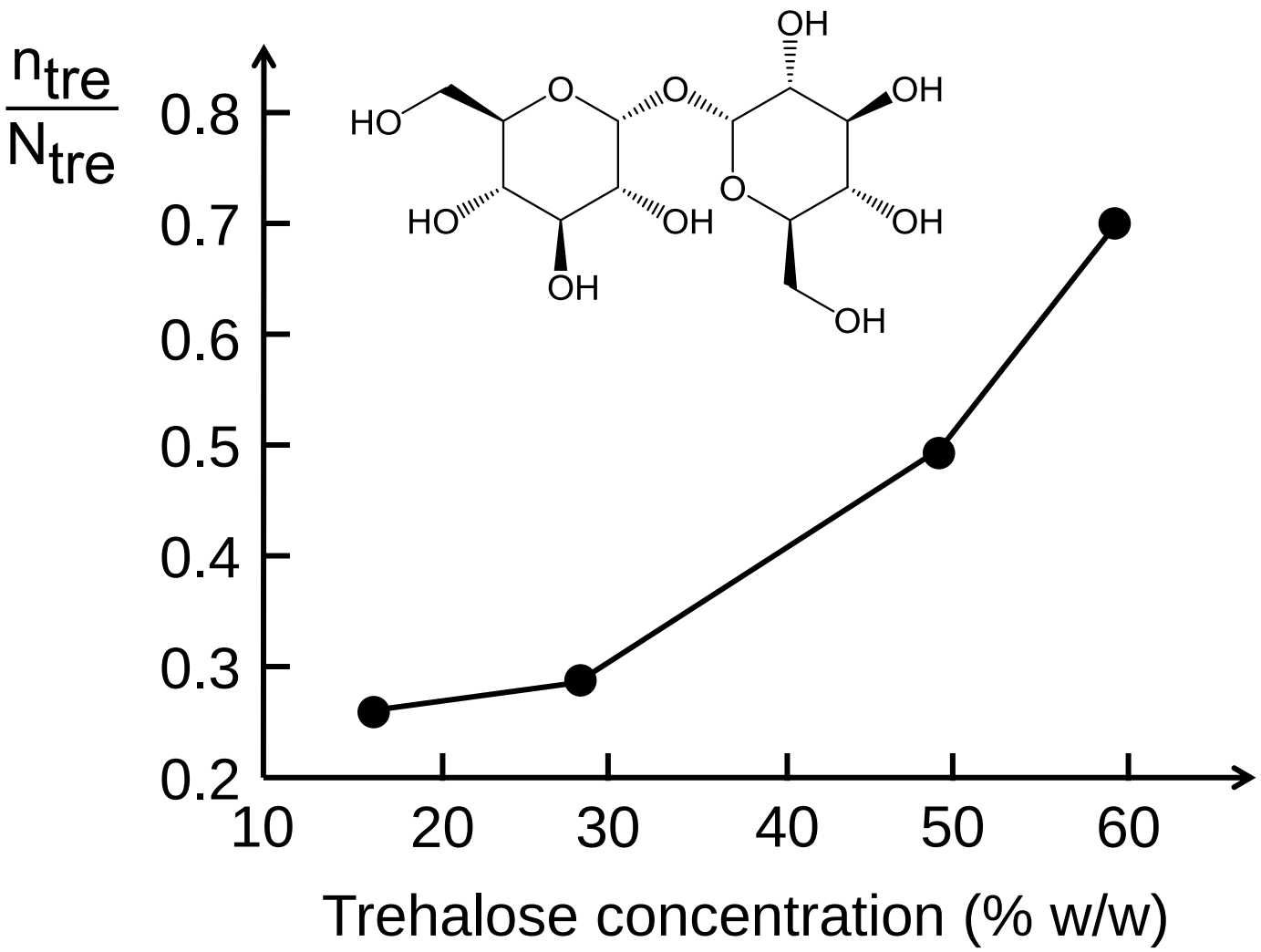
Monomer Dimer Trimer

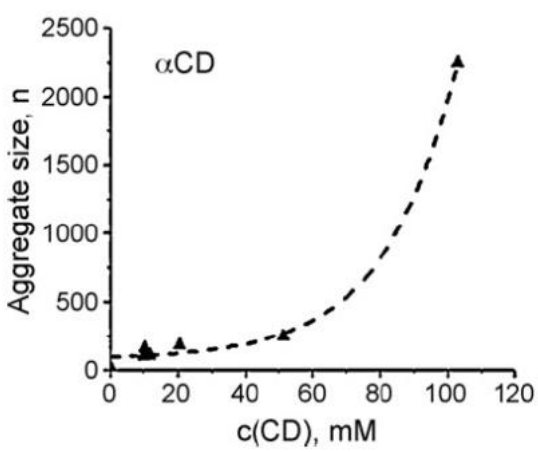
Higher order oligomers

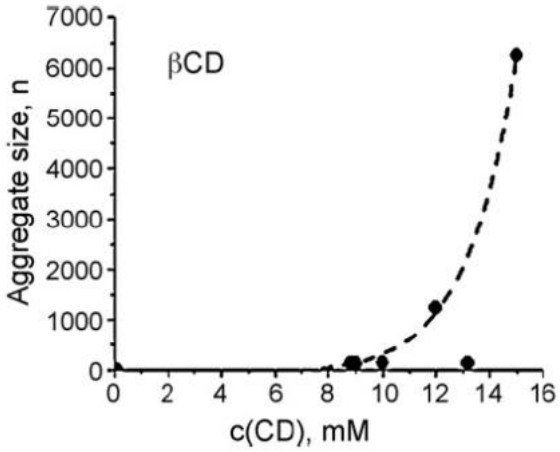


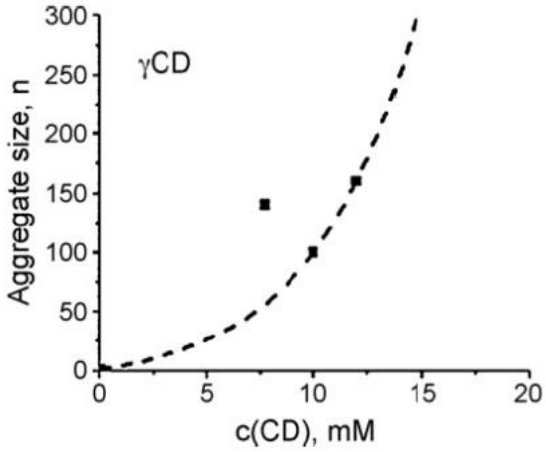

Shared water molecules
2
1.5
1
0.5
0
-0.5
8
10
12
14
16
Distance between solute molecules [Å]


Glucose (20°C)
HPβCD (22°C)
Viscosity (cP)
0
20
40
60
80
0
20
40
60
80
Concentration (% w/v)

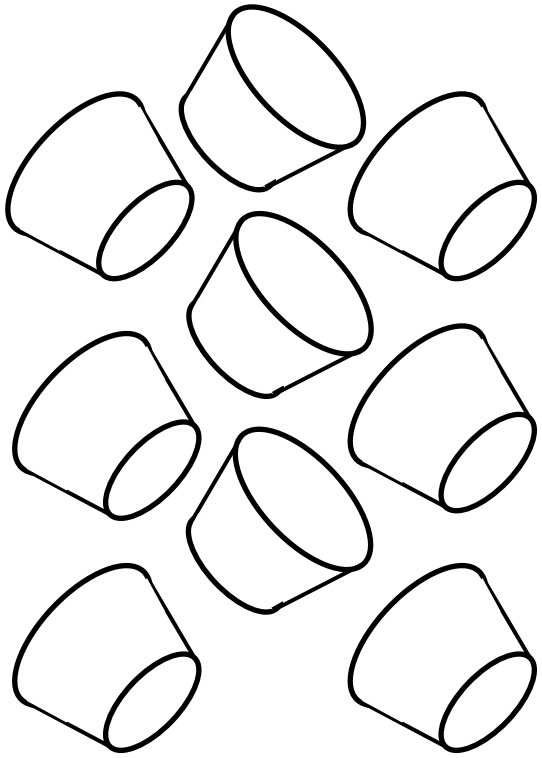

$\frac{n_{tre}}{N_{tre}}$
0.8
0.7
0.6
0.5
0.4
0.3
0.2
10
20
30
40
50
60
Trehalose concentration (% w/w)
HO
O
O
OH
OH
HO
OH
OH
O
OH
OH
OH


αCD
Aggregate size, n
c(CD), mM
0
500
1000
1500
2000
2500
0
20
40
60
80
100
120

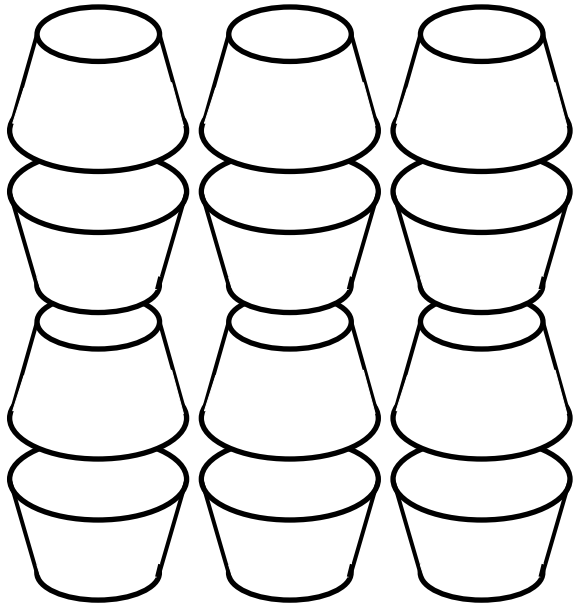

βCD
Aggregate size, n
c(CD), mM
0
1000
2000
3000
4000
5000
6000
7000
0
2
4
6
8
10
12
14
16

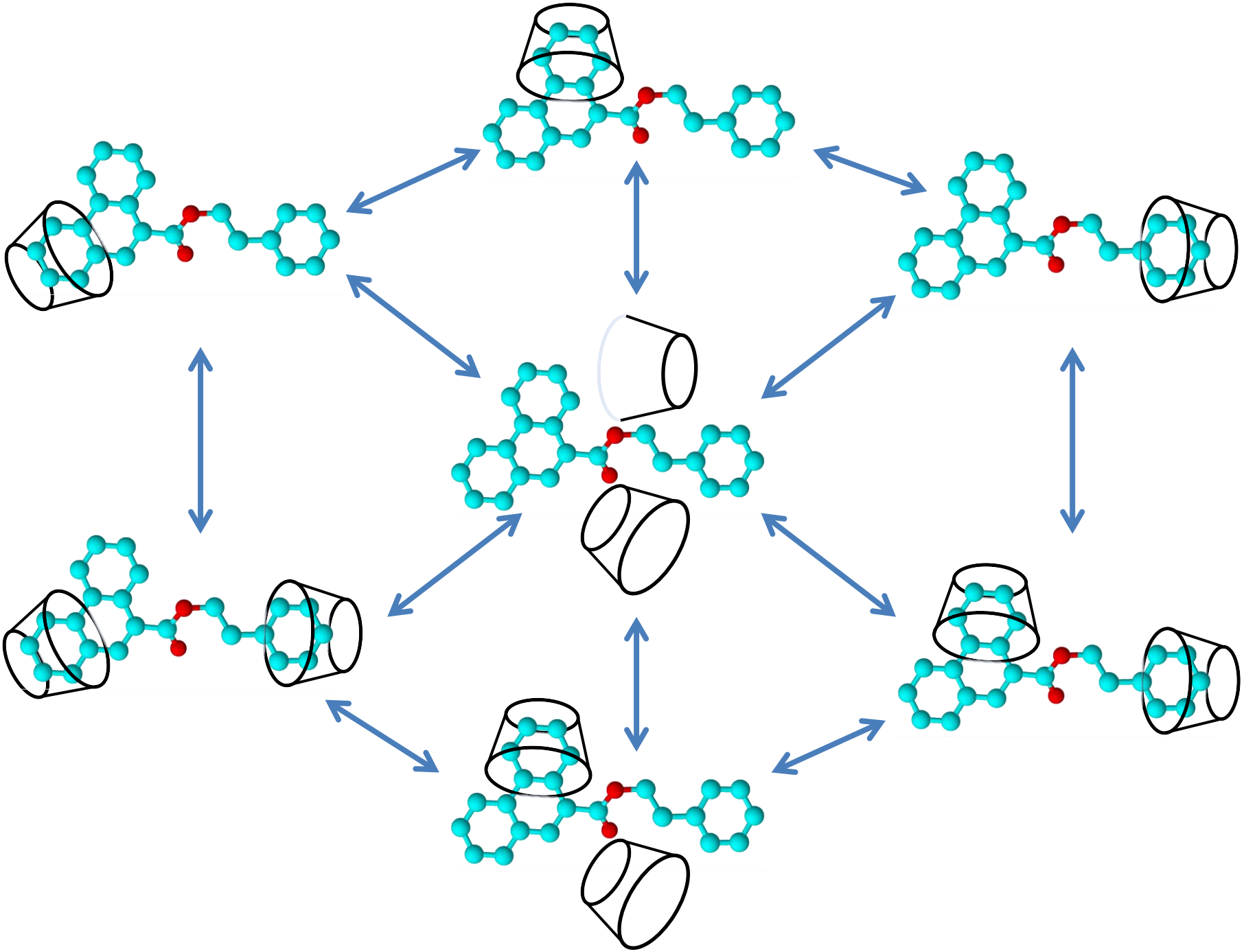

γCD
Aggregate size, n
c(CD), mM
0
50
100
150
200
250
300
0
5
10
15
20

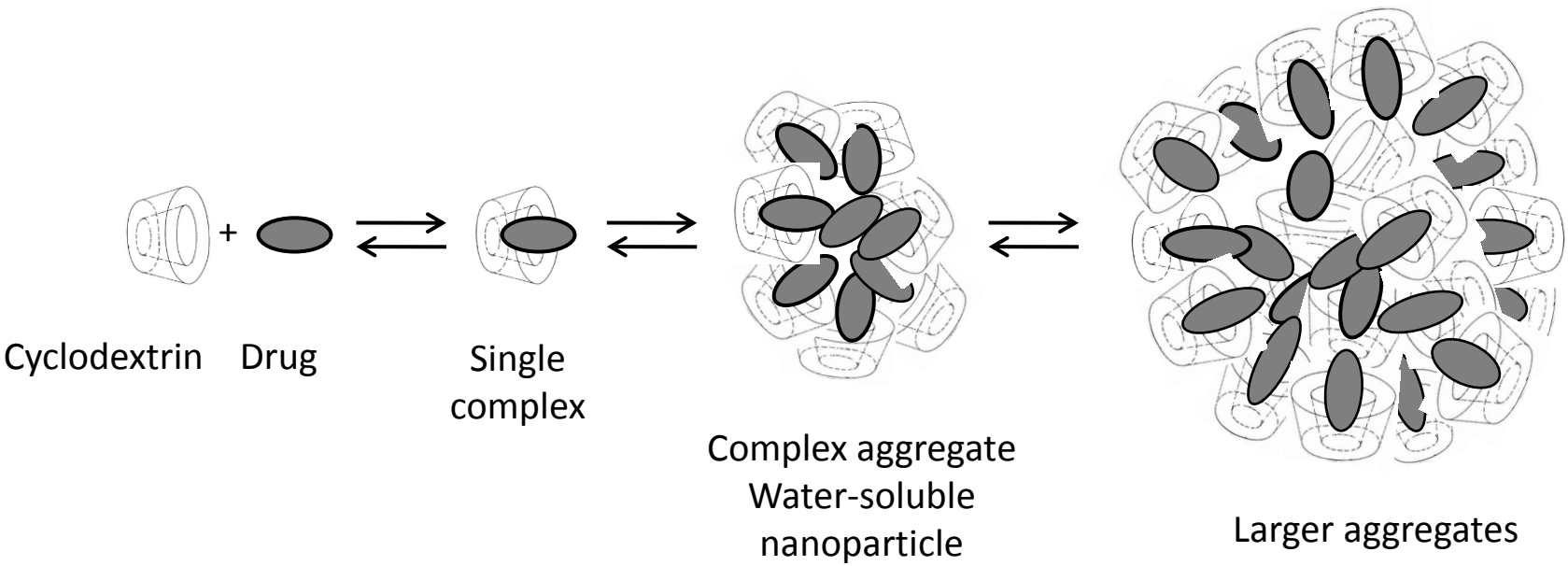
a.



b.






+
Cyclodextrin
Drug
Single complex
Complex aggregate
Water-soluble nanoparticle
Larger aggregates